



## TECHNICAL MEMORANDUM

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**Date:** 4 March 2011

**Project No.:** 10393351.001

**To:** Bob Medler, Bob Marriam (Remedium)

**Company:** Remedium

**From:** Sue Robinson

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### RE: RECOMMENDATIONS FOR BIRD PHYSIOLOGIST

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In July 2010, the Libby Asbestos Site Biological Technical Assistance Group (BTAG) met to discuss existing and potential future site work. Because the avian lung structure is different from that of mammals, EPA (Region 8) expressed an interest in identifying an independent expert on avian pulmonary physiology to advise them on the potential sensitivity of wild birds to environmental asbestos exposure that may occur at Operable Unit 3 of the Libby Asbestos Superfund Site. Remedium's Consultant Team was asked to provide recommendations of such experts for EPA's consideration.

Golder conducted a literature search with the goal of identifying, if possible, one or more well-published researchers in avian pulmonary physiology, preferably one with expertise related to impacts from particulate inhalation. To supplement the literature evaluation and broaden the overall search, numerous poultry association websites were identified and inquiries made seeking referrals and/or recommendations on avian pulmonary physiologists. This memorandum summarizes the methods and findings from this assessment and provides recommendations regarding avian physiologists that could provide the required support to EPA.

## 1.0 LITERATURE SEARCH

Several literature searches were performed in scientific literature databases using the following key words: ***bird, avian, broiler, chicken AND pulmonary, respiratory, asbestos, particulates, inhalation.*** The databases searched included: Agricola, Greenfile, Wildlife and Ecology Worldwide, Arctic and Antarctic Regions, Academic Search and Research and Development, BioOne, Compendex, Georef, Geobase, Scirus, Medline, Toxline, Biosis Previews, and general Google and Google Scholar searches.

The literature database results are summarized by author and their current location in Table 1. Researchers in the United States and Canada are separated within the table from those working in other parts of the globe, since it is desirable to find a specialist in the US that would work with the EPA. General internet searches for identified US and Canadian authors were performed to confirm whether the researcher is still involved in the area of study identified through the literature search. Where this information could be determined, it is summarized in the table columns 'Current Location' and 'Current Status'. A number of retired and professor emeritus status individuals were identified from this exercise.

Overall, 47 studies were identified dealing with research on some aspect of avian inhalation and pulmonary issues at various locations around the globe. There was one seminal paper found titled 'Asbestos-induced tumors in white leghorn fowls' published by researchers in Scotland in 1965. However, P.R. Peacock has not published since 1970 and A. Peacock is publishing in pulmonary issues in humans and is not an avian expert. The majority of the recent relevant literature focused on effects in chickens from dust. The most published authors in this area are Joseph D. Brain at Harvard School of Public Health, M.R. Fedde (Professor Emeritus) at Kansas State University, Judit E. Smits at the University of Saskatchewan, and R.F. Wideman, Jr. at the University of Arkansas.

## 2.0 CONTACTS WITH POULTRY ASSOCIATIONS

Poultry associations were researched and attempts made to contact association representatives for possible referrals. Poultry associations and trade organizations are listed comprehensively on the West Virginia University Extension Service Website (<http://www.wvu.edu/~agexten/poultry/assoc.htm>). The following organizations were contacted:

- United States Egg and Poultry Association
- Poultry Science Association
- National Contract Poultry Growers Association
- National Broiler Council
- National Turkey Federation
- American Egg Board
- Southeastern Poultry Research Lab
- USDA Poultry Reports and Databases
- U.S.A. Poultry and Egg Export Council
- Animal Agricultural Alliance
- Chicken Farmers of Canada
- World's Poultry Science Association
- United Egg Association
- Egg Clearing House

Some of the organizations were lobbying sites dedicated to the promotion of products and yielded no applicable information. However, upon contacting the above organizations, eleven (11) possible leads were identified and contacted. Responses (where received) follow.

Contacts:

- U.S. Poultry and Egg Association: referred us to the University of Georgia
- Dr. Johnson 'pjohnson@nifa.usda.gov': referral to Dr. Bottje
- Poultry Science Association 'psa@assochq.org': no response
- USDA 'ace@aphis.usda.gov': no response
- USDA 'acwest@aphis.usda.gov': referral to Dr. Pabilania
- USDA Scott.Branton@ars.usda.gov: no response

### 3.0 CALLS TO POTENTIAL EXPERTS

- Dr. Pabilania Colorado State University 'Kristy.Pabilonia@ColoState.EDU': responded. She is interested in veterinary sciences and not an expert in lung function.
- Dr. Pickrell University of Illinois-Urbana: out on indefinite medical leave.
- Dr. Kiepper University of Georgia 'bkiepper@uga.edu': no response
- Dr. Dunkley University of Georgia 'cdunkley@uga.edu': referred us to Dr. Casey Ritz
- Dr. Bottje University of Arkansas 'wbottje@uark.edu': referred us to Dr. Bob Wideman
- Dr. Casey Ritz [critz@uga.edu](mailto:critz@uga.edu); recommended Dr. Wideman.
- Dr Darryl Heard: replied in the negative (i.e., not an expert) with no referrals.
- Dr. Bob Wideman, University of Arkansas. [rwideman@uark.edu](mailto:rwideman@uark.edu); strong background in avian (poultry) physiology and pulmonary issues. Several publications provided by him directly.

The following potential experts were not contacted as noted below:

- Tracy Clippinger is on faculty at the Veterinary School at UC Davis. She published several papers on clinical diseases in birds during her residency in Zoo Animal Medicine at University of Florida and the San Diego Zoo, but is not an avian pulmonary specialist. She a general practitioner in zoo and wildlife medicine.
- James K. Morrissey at the Cornell Veterinary College wrote a review paper on "Diseases of the avian upper respiratory tract" in 1997, but has not pursued this as an area of research since then.

Of all the people contacted, Dr. Wideman appears to have the strongest background in avian pulmonary physiology. Furthermore, he was recommended by two of those independently contacted: Drs Bottje and Ritz. Dr. Wideman is well published and upon contact provided several of his papers on various research topics on pulmonary problems in broilers. Dr. Wideman self identifies as an avian physiologist with avian pulmonary expertise. He has been working in the field since the 1970's when most seminal avian physiology work and research was conducted. He indicates he is one of the only remaining avian pulmonary specialists that continues to work in the field (he changed his focus to poultry science when grant funding for studying other types of birds dried up in the 1970's and 80's, and now routinely conducts his research with broilers). In sum, Dr. Wideman is well published and is a recognized expert in the field of avian pulmonary physiology. A complete summary of his credentials is attached to this memorandum.

Dr. Wideman identified a Dr. Richard E. Brown, formerly of the Department of Environmental Health, Harvard School of Public Health as another potential expert. Dr. Brown's seminal publication according to Dr. Wideman is: The avian respiratory system: a unique model for studies of respiratory toxicosis and for monitoring air quality (Environmental Health Perspectives, 1997, 105:188-200). However, according to Dr. Wideman, Dr. Brown is no longer working in this field. Google searches did not identify Dr. Brown's current location (he does not appear to be at Harvard any longer) so may no longer be practicing research in avian pulmonary effects.

#### 4.0 OTHER

The databases listed on the Whole Wildlife Toxicology Catalog website (<http://www.pwrc.usgs.gov/wwtc/>) were reviewed for effects of particulates in avian pulmonary systems. No applicable information on asbestos (or silicosis, silica, dust) and birds was identified. An entry for asbestos in the Hazardous Substances Data Bank (<http://toxnet.nlm.nih.gov/cgi-bin/sis/search/r?dbs+hsdb:@term+@rn+1332-21-4>) had a number of mammalian studies referenced, but no avian studies.

#### 5.0 RECOMMENDATIONS

The literature database search identified a number of researchers located around the globe that have published articles relating to pulmonary effects of particulates in birds. The most published authors in North America in this area are Joseph D. Brain at Harvard School of Public Health, M.R. Fedde (Professor Emeritus) at Kansas State University, Judit E. Smits at the University of Saskatchewan, and R.F. Wideman, Jr. at the University of Arkansas. Dr. Brain was not contacted directly but is on the EPA Science Advisory Board. His research emphasizes the body's responses to inhaled particulates and gases and appears to be human health focused (i.e., using animal models). His biological sketch is available on EPA's Science Advisory Board Staff website<sup>1</sup>.

Of the researchers discovered, Dr. Robert Wideman has the strongest qualifications given both his research regarding avian pulmonary function (where he continues his research today) and the independent recommendation of his avian pulmonary expertise by two different expert researchers contacted. Dr. Wideman is an avian physiologist with pulmonary expertise and routinely conducts research in poultry (broilers) evaluating function and effects of stressors thereon, including on the pulmonary system. Accordingly, Dr. Wideman would be an excellent candidate to support EPA. He can be contacted at: [rwideman@uark.edu](mailto:rwideman@uark.edu). Golder has attached Dr. Wideman's CV and several relevant recent publications to this memorandum.

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<sup>1</sup> <http://yosemite.epa.gov/sab/sabpeople.nsf/WebPeople/BrainJoseph%20D.?OpenDocument>

## TECHNICAL MEMORANDUM

**TABLE 1**  
**Summary of Literature Search Findings**

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
<b>US or Canadian Authors</b>						
Abraham, J.	SUNY Upstate Medical University	Current in environmental and occupational pulmonary diseases	1	Comparative pathology of silicate pneumoconiosis.	1979. Am J Pathol. 96(1): 149-70.	Observations of simple pneumoconiosis of animals at the San Diego Zoo.
Barnas, G.M.	NIH Center for Scientific Review	Current	1	Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, <i>Anas platyrhynchos</i> .	1987. Respir Physiol. 67(1)22-36.	Inhaled aerosol particles
Benirschke, K.	UCSC, Department of Pathology	Professor emeritus	1	Comparative pathology of silicate pneumoconiosis.	1979. Am J Pathol. 96(1): 149-70.	Observations of simple pneumoconiosis of animals at the San Diego Zoo.
Besch, E.L.	University of Florida, presumed retired	Presumed retired	1	Airborne-particle deposition in the respiratory tract of chickens	1974. Poult Sci. 53(4): 1507-11.	Study involved latex particles inhaled by chickens to determine distribution in respiratory system.
Bishop, C.A.	Environment Canada Science and Technology Branch	Current, studies anthropogenic impacts on reptiles, amphibians, and birds	1	Pulmonary Histopathology in Ring-Billed Gulls ( <i>Larus delawarensis</i> ) from Colonies near Steel Mills and in Rural Areas.	Bulletin of Environmental Contamination & Toxicology May2001, Vol. 66 Issue 5, p563-569, 7p	No histopathological changes after 2-3 months of exposure to releases from steel mills
Bland, M.C.	UC Davis School of Vet Med.	Current as of 2009	1	Duration of exposure--histological effects on broiler lungs, performance, and house environment with Mt. St. Helens' volcanic ash dust	1985. Poult Sci. 64(1): 51-8.	Broilers exposed to Mt. St. Helen's volcanic ash dust from 28 to 49 days of age to measure effects on lung tissue, body weight, and mortality

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Bloor, C.	UCSC, Department of Pathology	Professor emeritus	1	Comparative pathology of silicate pneumoconiosis.	1979. Am J Pathol. 96(1): 149-70.	Observations of simple pneumoconiosis of animals at the San Diego Zoo.
Boardman, M.L.	UC Davis	Unknown, assumed retired	1	Relationship of pulmonary particulates in English sparrows to gross air pollution	1974. Journal of Wildlife Diseases. 10(4):335-340.	Study of this species as an indicator of atmospheric pollutants
Bortolotti, Gary	University of Saskatchewan, Canada	Current professor of avian biology	1	Effects of inhalation exposure to a binary mixture of benzene and toluene on vitamin A status and humoral and cell-mediated immunity in wild and captive American kestrels	Journal of Toxicology and Environmental Health Part A; 2008. 71(16): p1100-1108.	Benzene and toluene testing of kestrels
Bottje, W.G.	University of Arkansas	Professor of physiology in poultry science	1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid
Brain, Joseph D.	Harvard University Medical School	Professor of environmental physiology	3	The avian respiratory system: A unique model for studies of respiratory toxicosis and monitoring for air quality	Environmental Health Perspectives; Feb1997, Vol. 105 Issue 2, p188	General discussion regarding avian respiratory system and exposure to gases and airborne particulates
Brain, Joseph D.	Harvard School of Public Health	Professor of environmental physiology	3	Deposition and clearance of inhaled aerosol in the respiratory tract of chickens	1982. Journal of Applied Physiology. 53(6):1423-1428.	16 chickens were exposed to an aerosol of submicrometric particles and the clearance rate observed over 36 hrs.
Brain, Joseph D.	Harvard School of Public Health	Professor of environmental physiology	3	Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, <i>Anas platyrhynchos</i> .	1987. Respir Physiol. 67(1)22-36.	Inhaled aerosol particles
Brown, Richard E.	unknown (previously at Harvard Medical School)	Unknown	1	The avian respiratory system: A unique model for studies of respiratory toxicosis and monitoring for air quality	Environmental Health Perspectives; Feb1997, Vol. 105 Issue 2, p188	General discussion regarding avian respiratory system and exposure to gases and airborne particulates

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Clippinger, Tracy L.	San Diego Zoo	Current veterinarian	1	Diseases of the lower respiratory tract of companion birds	1997. Seminars in Avian and Exotic Pet Medicine. 6(4): 201-208	Overview of diseases and treatments
Fedde, M. R.	Kansas State U.	Professor emeritus	5	Ventilatory Sensitivity to Changes in Inspired and Arterial Carbon Dioxide Partial Pressures in the Chicken	Poult Sci (2002)	
Fedde, M. R.	Kansas State U.	Professor emeritus	5	Influence of Feed Deprivation on Ventilation and Gas Exchange in Broilers: Relationship to Pulmonary Hypertension Syndrome	Poult Sci (1998)	
Fedde, M. R.	Kansas State U.	Professor emeritus	5	Cardio-Pulmonary Function in Preascitic (Hypoxemic) or Normal Broilers Inhaling Ambient Air or 100% Oxygen	2000 Poultry Science 79: 415-425	
Fedde, M.R.	Kansas State U.	Professor emeritus	5	Relationship of structure and function of the avian respiratory system to disease susceptibility	1998. Poultry Science. 77(8):1130-1138.	Discussion of avian respiratory system and inhaled foreign particles
Fedde, M.R.	Kansas State U.	Professor emeritus	5	Respiration	1986. Pages 191-220 in: Avian Physiology. 4th Ed. PD Sturkie, Ed. Springer-Verlag, NY.	Book chapter
Foutz, T.L.	University of Georgia	Faculty in the Department of Biological and Agricultural Engineering	1	Respirable tissue damage in broilers exposed to aerosol particles and ammonia	Transactions of the ASAE	Broilers exposed to respirable aerosols and noxious gasses, effects on weight gain and mortality for 7 weeks

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Goeger, M.P.	Oregon State University	Presumed retired	1	Duration of exposure-- histological effects on broiler lungs, performance, and house environment with Mt. St. Helens' volcanic ash dust	1985. Poult Sci. 64(1): 51-8.	Broilers exposed to Mt. St. Helen's volcanic ash dust from 28 to 49 days of age to measure effects on lung tissue, body weight, and mortality
Hayter, R.B.	Kansas State U.	Associate Dean of Engineering	1	Airborne-particle deposition in the respiratory tract of chickens	1974. Poult Sci. 53(4): 1507-11.	Study of latex particles inhaled by chickens to determine distribution in respiratory system.
Heard, Darryl J.	University of Florida	Associate Professor	1	Avian respiratory anatomy and physiology	1997. Seminars in Avian and Exotic Pet Medicine. 6(4): 172-179	Overview of avian respiratory anatomy and physiology
Helfer, D.H.	Oregon State University	Presumed retired (all publications prior to 1985)	1	Duration of exposure-- histological effects on broiler lungs, performance, and house environment with Mt. St. Helens' volcanic ash dust	1985. Poult Sci. 64(1): 51-8.	Broilers exposed to Mt. St. Helen's volcanic ash dust from 28 to 49 days of age to measure effects on lung tissue, body weight, and mortality
Kuhlmann, W. D.	Kansas State U.	Believed to be retired	1	Ventilatory Sensitivity to Changes in Inspired and Arterial Carbon Dioxide Partial Pressures in the Chicken	Poult Sci (2002)	Not currently on KSU Staff or emeritus list
McArn, G.E.	UC Davis	Unknown, assumed retired	1	Relationship of pulmonary particulates in English sparrows to gross air pollution	1974. Journal of Wildlife Diseases. 10(4):335-340.	Study of this species as an indicator of atmospheric pollutants
Mensah, George A.	PepsiCo	Has worked for CDC and Harvard Center for Public Health	1	Deposition and clearance of inhaled aerosol in the respiratory tract of chickens	1982. Journal of Applied Physiology. 53(6):1423-1428.	16 chickens were exposed to an aerosol of submicrometric particles and the clearance rate observed over 36 hrs.
Morrissey, James K.	Cornell University, NY	Lecturer in Exotic and Wildlife Medicine	1	Diseases of the upper respiratory tract of companion birds	1997. Seminars in Avian and Exotic Pet Medicine. 6(4): 195-200	Overview of diseases and treatments

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Munn, R.	UC Davis	Assumed retired (all published articles prior to 1980)	1	Relationship of pulmonary particulates in English sparrows to gross air pollution	1974. Journal of Wildlife Diseases. 10(4):335-340.	Study of this species as an indicator of atmospheric pollutants
Nakaeu, H.S	Unknown (previously at Oregon State U.)	Unknown	2	Duration of exposure-- histological effects on broiler lungs, performance, and house environment with Mt. St. Helens' volcanic ash dust	1985. Poult Sci. 64(1): 51-8.	Broilers exposed to Mt. St. Helen's volcanic ash dust from 28 to 49 days of age to measure effects on lung tissue, body weight, and mortality
Nakaeu, H.S	Unknown (previously at Oregon State U.)	Unknown	2	Effects of Mt. St. Helen's volcanic ash on broiler performance and health and on house environment.	1982. Poult Sci. 61: 693-698	Effects of ash on broiler performance
Nelson, P. I.	Kansas State U.	Professor of Statistics	1	Ventilatory Sensitivity to Changes in Inspired and Arterial Carbon Dioxide Partial Pressures in the Chicken	Poult Sci (2002)	
Newman, J.R.	Unknown (previously at Environmental Science and Engineering in Gainesville FL)	Unknown	1	Effects of air emissions on wildlife resources	Book: Air pollution and acid rain report No. 1; May 1980; 43 pp.	Book review of contaminants and wildlife, asbestos is a subject term
Olsgard, Mandy L.	University of Saskatchewan, Canada	Toxicology Centre	2	Effects of inhalation exposure to a binary mixture of benzene and toluene on vitamin A status and humoral and cell-mediated immunity in wild and captive American kestrels	Journal of Toxicology and Environmental Health Part A; January 2008, Vol. 71 Issue: Number 16 p1100-1108	Benzene and toluene testing of kestrels
Olsgard, Mandy L.	University of Saskatchewan, Canada	Toxicology Centre	2	The design, construction, and operation of a whole-body inhalation chamber for use in avian toxicity studies	2008. Inhalation Toxicology. 20:191-197.	Design for a chamber for gasses, references for toxicity of particulates (oil and gas emissions)

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Pickrell, J.A.	Kansas State U.	Currently Associate Professor of Environmental Toxicology	1	Chapter 12 - Respiratory toxicity	Veterinary toxicology. 2007. pp177-192	Current research in swine. Book chapter review of respiratory toxicity.
Poss, P.E.	College of Veterinary Medicine, University of Minnesota	Presumed retired (all publications prior to 2001)	1	Turkey industry strategies for control of respiratory and enteric diseases	1998. Poultry Science. 77:1181-1185.	Review paper discussing strategies to control respiratory diseases
Quinn, J.S.	McMaster University, Canada	Professor of Biology	1	Pulmonary Histopathology in Ring-Billed Gulls (Larus delawarensis) from Colonies near Steel Mills and in Rural Areas.	Bulletin of Environmental Contamination & Toxicology May2001, Vol. 66 Issue 5, p563-569, 7p	No histopathological changes after 2-3 months of exposure to releases from steel mills
Rowland, G.N	University of Georgia	Presumed retired (publications prior to 2007 and not listed on UGS website)	1	Respirable tissue damage in broilers exposed to aerosol particles and ammonia	2001. Transactions of the ASAE	Broilers exposed to respirable aerosols and noxious gasses, effects on weight gain and mortality for 7 weeks
Smits, Judit E.	University of Saskatchewan, Canada	Toxicology Centre	3	Effects of inhalation exposure to a binary mixture of benzene and toluene on vitamin A status and humoral and cell-mediated immunity in wild and captive American kestrels	Journal of Toxicology and Environmental Health Part A; January 2008, Vol. 71 Issue: Number 16 p1100-1108	Benzene and toluene testing of kestrels
Smits, Judit E.	University of Saskatchewan, Canada	Toxicology Centre	3	The design, construction, and operation of a whole-body inhalation chamber for use in avian toxicity studies	2008. Inhalation Toxicology. 20:191-197.	Design for a chamber for gasses, references for toxicity of particulates (oil and gas emissions)

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Smits, Judit E.	University of Saskatchewan, Canada	Toxicology Centre	3	Pulmonary Histopathology in Ring-Billed Gulls ( <i>Larus delawarensis</i> ) from Colonies near Steel Mills and in Rural Areas.	Bulletin of Environmental Contamination & Toxicology May2001, Vol. 66 Issue 5, p563-569, 7p	No histopathological changes after 2-3 months of exposure to releases from steel mills
Stearns, R.C.	Harvard School of Public Health	Technical Director, Electron Microscopy Lab	1	Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, <i>Anas platyrhynchos</i> .	1987. Respir Physiol. 67(1)22-36.	Inhaled aerosol particles
Tackett, C. D.	University of Arkansas	Presumed retired (publications prior to 2000 and not listed on U Ark website)	1	Cardio-Pulmonary Function in Preascitic (Hypoxemic) or Normal Broilers Inhaling Ambient Air or 100% Oxygen	2000 Poultry Science 79: 415-425	
Taylor, Michael	University of Guelph, Canada	Teaching faculty at the Ontario Veterinary College	1	Endoscopic diagnosis of avian respiratory tract diseases	1997. Seminars in Avian and Exotic Pet Medicine Volume 6, Issue 4: 187-194	Approaches of avian endoscopy
Trask, Brenda	University of Saskatchewan, Canada	Department of Veterinary Pathology	1	Effects of inhalation exposure to a binary mixture of benzene and toluene on vitamin A status and humoral and cell-mediated immunity in wild and captive American kestrels	Journal of Toxicology and Environmental Health Part A; January 2008, Vol. 71 Issue: Number 16 p1100-1108	Benzene and toluene testing of kestrels
Van Wicklen, G.L.	University of Delaware	No longer on staff	1	Respirable tissue damage in broilers exposed to aerosol particles and ammonia	Transactions of the ASAE	Broilers exposed to respirable aerosols and noxious gasses, effects on weight gain and mortality for 7 weeks

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Wang, Ning	University of Illinois	Professor of Mechanical Science and Engineering	1	The avian respiratory system: A unique model for studies of respiratory toxicosis and monitoring for air quality	Environmental Health Perspectives; Feb1997, Vol. 105 Issue 2, p188	General discussion regarding avian respiratory system and exposure to gases and airborne particulates
Wang, S.	Unknown (previously at the University of Arkansas)	Unknown	1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid
Weigle, G. E.	Kansas State U.	Departments of Anatomy and Physiology	2	Influence of Feed Deprivation on Ventilation and Gas Exchange in Broilers: Relationship to Pulmonary Hypertension Syndrome	Poult Sci (1998)	Cannot determine current status, believed to be retired
Weigle, G. E.	Kansas State U.	Departments of Anatomy and Physiology	2	Cardio-Pulmonary Function in Preascitic (Hypoxemic) or Normal Broilers Inhaling Ambient Air or 100% Oxygen	2000 Poultry Science 79: 415-425	
Wellings, S.R.	UC Davis	Retired in April 2010	1	Relationship of pulmonary particulates in English sparrows to gross air pollution	1974. Journal of Wildlife Diseases. 10(4):335-340.	Study of this species as an indicator of atmospheric pollutants
Wideman Jr., R. F.	University of Arkansas	Currently Associate Director of the Center and Physiologist	3	Influence of Feed Deprivation on Ventilation and Gas Exchange in Broilers: Relationship to Pulmonary Hypertension Syndrome	Poult Sci (1998)	
Wideman Jr., R. F.	University of Arkansas	Currently Associate Director of the Center and Physiologist	3	Cardio-Pulmonary Function in Preascitic (Hypoxemic) or Normal Broilers Inhaling Ambient Air or 100% Oxygen	2000 Poultry Science 79: 415-425	

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Wideman Jr., R. F.	University of Arkansas	Currently Associate Director of the Center and Physiologist	3	Differential expression of vasoactive mediators in microparticle-challenged lungs of chickens that differ in susceptibility to pulmonary arterial hypertension.	2010. Regu. Physiol. 298(1):R235-R242.	
Williams, A.	University of Arkansas	Assumed retired (not on faculty or staff list)	1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid
<b>Authors Currently Residing Abroad</b>						
Aarnink, A.J.A	Netherlands		1	Effects of dust and airborne dust components on antibody responses, body weight gain, and heart morphology of broilers	2009. Poultry Science. 88:1838-1849	Chickens dosed intratracheally with dust and other components to determine effect on BW and heart morphology
Adekunle, J.S.	South Africa		1	Comparative in vitro study of interactions between particles and respiratory surface macrophages, erythrocytes, and epithelial cells of the chicken and the rat.	Journal of Anatomy; Oct2008, Vol. 213 Issue 4, p452-463	Polystyrene exposure of chickens and rats, study of lung protection systems
Borzacchiello, G.	Italy		1	Silicate Pneumoconiosis in hens	2000. Journal of comparative pathology. 122(4):249-254.	Observations of 13 cases of silicate pneumo. In 3-4 year old hens in Italy.
Brackenbury, JH	England		1	Ventilation of the lung-air sac system.	1987. in: Bird Respiration. Vol. 1. JT Seller, ed. Pgs 39-69. CRC Press, Boca Raton, FL.	Book chapter review of respiratory function of birds
Brambilla, C.	France		1	Comparative pathology of silicate pneumoconiosis.	1979. Am J Pathol. 96(1): 149-70.	Observations of simple pneumoconiosis of animals at the San Diego Zoo.

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Brambilla, E.	France		1	Comparative pathology of silicate pneumoconiosis.	1979. Am J Pathol. 96(1): 149-70.	Observations of simple pneumoconiosis of animals at the San Diego Zoo.
Dalamani, G.	Germany		3	The avian lung-associated immune system: a review	2006. Veterinary Research. 37:311-324.	Physiological review of the immune system component of the avian lung
Dalamani, G.	Germany		3	Ontogenesis of the Chicken Bronchus Associated Lymphoid Tissue (BALT)	Anatomia, Histologia, Embryologia v. 34, Issue Supplement s1, pg41, Dec. 2005	
Dalamani, G.	Germany		3	The lung associated immune system of the chicken	International Symposium on Avian corona and pneumovirus infections, Rauscholzhausen, Germany, 20-23 June 2004	Conference paper
Duncker, Hans-Rainer	Germany (assumed)		1	Der Atemapparat der Vögel und ihre lokomotorische und metabolische Leistungsfähigkeit (The respiratory apparatus of birds and their locomotor and metabolic performance)	Journal für Ornithologie; January 2000, Vol. 141 Issue: Number 1 p1-67	Summary of the respiratory system of birds, comparison between poultry and flying birds
Dunster, C.	Lab Supervisor, King's College, London, UK		1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Durfort, M.	Spain		2	A study of ciliar tracheal epithelium on passerine birds and small mammals subjected to air pollution: Ultrastructural study	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 27 Issue: Number 1 p137-142	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO2, NOx, and particulates)- focus on the ciliar tracheal epithelium
Durfort, M.	Spain		2	Effects of air pollution on passerine birds and small mammals	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 24 Issue: Number 1 p59-66	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO2, NOx, and particulates)
Galati, P.	Italy		1	Silicate Pneumoconiosis in hens	2000. Journal of comparative pathology. 122(4):249-254.	Observations of 13 cases of silicate pneumo. In 3-4 year old hens in Italy.
Gorriz, A.	Spain		2	A study of ciliar tracheal epithelium on passerine birds and small mammals subjected to air pollution: Ultrastructural study	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 27 Issue: Number 1 p137-142	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO2, NOx, and particulates) - focus on the ciliar tracheal epithelium
Gorriz, A.	Spain		2	Effects of air pollution on passerine birds and small mammals	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 24 Issue: Number 1 p59-66	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO2, NOx, and particulates)
Homidan, A.A.	Saudi Arabia		1	Review of the effect of ammonia and dust concentrations on broiler performance	2003. World's Poult. Sci. J. 59:340-349	Review paper

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Kaspers, B.	Germany		3	The avian lung-associated immune system: a review	2006. Veterinary Research. 37:311-324.	Physiological review of the immune system component of the avian lung
Kaspers, B.	Germany		3	Ontogenesis of the Chicken Bronchus Associated Lymphoid Tissue (BALT)	Anatomia, Histologia, Embryologia v. 34, Issue Supplement s1, pg41, Dec. 2005	
Kaspers, B.	Germany		3	The lung associated immune system of the chicken	International Symposium on Avian corona and pneumovirus infections, Rauscholzhausen, Germany, 20-23 June 2004	Conference paper
Kelly, F.J.	King's College, London	Professor of Environmental Health	1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid
Kemp, B.	Netherlands		1	Effects of dust and airborne dust components on antibody responses, body weight gain, and heart morphology of broilers	2009. Poultry Science. 88:1838-1849	Chickens dosed intratracheally with dust and other components to determine effect on BW and heart morphology
Kiama, S.G	Nairobi, Kenya		1	Comparative in vitro study of interactions between particles and respiratory surface macrophages, erythrocytes, and epithelial cells of the chicken and the rat.	Journal of Anatomy; Oct2008, Vol. 213 Issue 4, p452-463	Polystyrene exposure of chickens and rats, study of lung protection systems
King, A. S.	England		2	The Thickness of the avian blood-gas barrier: qualitative and quantitative observations	Journal of Anatomy (1992), 134, 3, pp. 553-562	

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
King, A.S.	England		2	Structural and functional aspects of the avian lungs and air sacs.	1966. Pages 171–267 in: International Review of General and Experimental Zoology. W.J.L. Felts and R. J. Harrison, ed. Academic Press, New York, NY.	
Kothlow, S.	Germany		2	Ontogenesis of the Chicken Bronchus Associated Lymphoid Tissue (BAL)	Anatomia, Histologia, Embryologia v. 34, Issue Supplement s1, pg41, Dec. 2005	
Kothlow, S.	Germany		2	The lung associated immune system of the chicken	International Symposium on Avian corona and pneumovirus infections, Rauscholzhausen, Germany, 20-23 June 2004	Conference paper
Lai, H.T.L.	Vietnam		1	Effects of dust and airborne dust components on antibody responses, body weight gain, and heart morphology of broilers	2009. Poultry Science. 88:1838-1849	Chickens dosed intratracheally with dust and other components to determine effect on BW and heart morphology
Llacuna, S.	Spain		2	A study of ciliar tracheal epithelium on passerine birds and small mammals subjected to air pollution: Ultrastructural study	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 27 Issue: Number 1 p137-142	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO <sub>2</sub> , NO <sub>x</sub> , and particulates)- focus on the ciliar tracheal epithelium

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Llacuna, S.	Spain		2	Effects of air pollution on passerine birds and small mammals	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 24 Issue: Number 1 p59-66	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO <sub>2</sub> , NO <sub>x</sub> , and particulates)
Maina, J.N.	South Africa		9	Comparative in vitro study of interactions between particles and respiratory surface macrophages, erythrocytes, and epithelial cells of the chicken and the rat.	Journal of Anatomy; Oct2008, Vol. 213 Issue 4, p452-463	Polystyrene exposure of chickens and rats, study of lung protection systems
Maina, J.N.	South Africa		9	Composite cellular defense stratagem in the avian respiratory system: functional morphology of the free (surface) macrophages and specialized pulmonary epithelia.	2002. Journal of Anatomy. 200(5):499-516.	Physiological assessment of the free respiratory macrophages of the lung - air sac systems of birds compared with the rat
Maina, J.N.	South Africa		9	Study of the structure of the air and blood capillaries of the gas exchange tissue of the avian lung by serial section three-dimensional reconstruction.	2008. Journal of Microscopy. 230(1):84-93.	Avian lung structure
Maina, J.N.	South Africa		9	The Thickness of the avian blood-gas barrier: qualitative and quantitative observations	Journal of Anatomy (1992), 134, 3, pp. 553-562	
Maina, J.N.	South Africa		9	A scanning electron microscopic study of the air and blood capillaries of the lung of the domestic fowl (Gallus domesticus)	Cellular and Molecular Live Sciences v. 38, n. 5, 614-616	

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Maina, J.N.	South Africa		9	A qualitative and quantitative study of the lung of an ostrich, <i>Struthio camelus</i>	J Exp Biol. 2001 Jul;204(PT13);2313-30	
Maina, J.N.	South Africa		9	A systematic study of the development of the airway (bronchial) system of the avian lung from days 3 to 26 of embryogenesis: a transmission electron microscopic study on the domestic fowl, <i>Gallus gallus variant domesticus</i>	Tissue and Cell, v. 35, iss. 5, Oct 2003, pp. 375-391	
Maina, J.N.	South Africa		9	Development, Structure, and function of a novel respiratory organ, the lung-air sac system of birds: to go where no other vertebrate has gone	Biological Reviews, v. 81, Iss. 4, p 545-579, Nov. 2006	
Maina, J.N.	South Africa		9	Spectacularly robust! Tensegrity principle explains the mechanical strength of the avian lung	Reparatory Physiology and Neurobiology, v. 155, Iss. 1, Jan 2007 p. 1-10	
Mudway, I.	King's College, London	Lecturer in Respiratory Toxicology	1	Antioxidant defenses in lung lining fluid of broilers: Impact of poor ventilation conditions	1998. Poultry Science. 77:516-552	Male broiler chickens exposed to high concentrations of dust and ammonia for 6-7 wk, measured antioxidant composition of lung lining fluid
Nadal, J.	Spain		2	A study of ciliar tracheal epithelium on passerine birds and small mammals subjected to air pollution: Ultrastructural study	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 27 Issue: Number 1 p137-142	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO2, NOx, and particulates)- focus on the ciliar tracheal epithelium

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Nadal, J.	Spain		2	Effects of air pollution on passerine birds and small mammals	Archives of Environmental Contamination and Toxicology; January 1993, Vol. 24 Issue: Number 1 p59-66	Tests of birds and small mammals exposed to emissions from coal-fired power plants (SO <sub>2</sub> , NO <sub>x</sub> , and particulates)
Nganpiep, L.N.	South Africa		1	Composite cellular defense stratagem in the avian respiratory system: functional morphology of the free (surface) macrophages and specialized pulmonary epithelia.	2002. Journal of Anatomy. 200(5):499-516.	Physiological assessment of the free respiratory macrophages of the lung - air sac systems of birds compared with the rat
Nieuwland, M.G.B	Netherlands		1	Effects of dust and airborne dust components on antibody responses, body weight gain, and heart morphology of broilers	2009. Poultry Science. 88:1838-1849	Chickens dosed intratracheally with dust and other components to determine effect on BW and heart morphology
Parmentier, H.K	Netherlands		1	Effects of dust and airborne dust components on antibody responses, body weight gain, and heart morphology of broilers	2009. Poultry Science. 88:1838-1849	Chickens dosed intratracheally with dust and other components to determine effect on BW and heart morphology
Peacock, A.	Scotland		1	Asbestos-induced tumors in white leghorn fowls.	1965. Ann N Y Acad Sci. 132(1): 501-6.	Study was asbestos fibers administered directly to lungs in fowl, observed tumor incidence
Peacock, P.R.	Scotland		1	Asbestos-induced tumors in white leghorn fowls.	1965. Ann N Y Acad Sci. 132(1): 501-6.	Study was asbestos fibers administered directly to lungs in fowl, observed tumor incidence

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Petchey, A.M.	United Kingdom		1	Review of the effect of ammonia and dust concentrations on broiler performance	2003. World's Poult. Sci. J. 59:340-349	Review paper
Reece, S.	Germany		3	Ontogenesis of the Chicken Bronchus Associated Lymphoid Tissue (BALT)	Anatomia, Histologia, Embryologia v. 34, Issue Supplement s1, pg41, Dec. 2005	
Reece, S.	Germany		3	The lung associated immune system of the chicken	International Symposium on Avian corona and pneumovirus infections, Rauscholzhausen, Germany, 20-23 June 2004	Conference paper
Reese, S.	Germany		3	The avian lung-associated immune system: a review	2006. Veterinary Research. 37:311-324.	Physiological review of the immune system component of the avian lung
Robertson, J.F.	United Kingdom		1	Review of the effect of ammonia and dust concentrations on broiler performance	2003. World's Poult. Sci. J. 59:340-349	Review paper
Roperto, F.	Italy		1	Silicate Pneumoconiosis in hens	2000. Journal of comparative pathology. 122(4):249-254.	Observations of 13 cases of silicate pneumo. In 3-4 year old hens in Italy.
Ungaro, R.	Italy		1	Silicate Pneumoconiosis in hens	2000. Journal of comparative pathology. 122(4):249-254.	Observations of 13 cases of silicate pneumo. In 3-4 year old hens in Italy.
Walski, M.	Polish Academy of Sciences, Warsaw, Poland		1	Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, <i>Anas platyrhynchos</i> .	1987. Respir Physiol. 67(1)22-36.	Inhaled aerosol particles

Author	Current Location	Current Status	# Citations	Article Title	Source	Comment
Woodward, J.D.	South Africa		1	Study of the structure of the air and blood capillaries of the gas exchange tissue of the avian lung by serial section three-dimensional reconstruction.	2008. Journal of Microscopy. 230(1):84-93.	Avian lung structure
Yauk, C.L.	United Kingdom		1	Pulmonary Histopathology in Ring-Billed Gulls ( <i>Larus delawarensis</i> ) from Colonies near Steel Mills and in Rural Areas.	Bulletin of Environmental Contamination & Toxicology May2001, Vol. 66 Issue 5, p563-569, 7p	No histopathological changes after 2-3 months of exposure to releases from steel mills

## **ATTACHMENTS**

## **CURRICULUM VITAE: Robert F. Wideman, Jr., Ph.D.**

### **EDUCATION**

University of Delaware, Newark, DE	1967-1971	B.A.	(Biology)
University of Connecticut, Storrs, CT	1973-1974	M.S.	(Zoology)
University of Connecticut, Storrs, CT	1974-1978	Ph.D.	(Physiology)

### **CAREER HISTORY**

U.S. Army	1LT MSC	1971-1973
University of Connecticut, Dept Physiol.	Graduate Assistant	1973-1978
University of Arizona College of Medicine	Postdoctoral Research	1978-1981
Penn State University Dept. Poultry Sci.	Assistant Professor	1981-1987
Penn State University Dept. Poultry Sci.	Associate Professor	1987-1991
Penn State University Dept. Poultry Sci.	Professor	1991-1993
University of Arkansas Dept. Poultry Sci.	Arkansas Poultry Fed. Chair	1993-1997
University of Arkansas Dept. Poultry Sci.	Professor	1997-2004
University of Arkansas Dept. Poultry Sci.	CEPS Associate Director	2004-present

### **HONORS AND AWARDS**

Phi Kappa Phi Honor Society, University of Connecticut Chapter, 1976.  
Sigma Xi, Penn State Chapter, 1985.  
Poultry Science Association Research Award, 1988.  
Gamma Sigma Delta, Penn State Chapter, 1990.  
National Chicken Council Broiler Research Award, 1999.  
University of Arkansas John W. White Research Award, 2002.  
ESCOP/ACOP Leadership Development Program, Class 14, 2004.  
Fellow of the Poultry Science Association, 2008.  
Convocation Speaker, Distinguished Alumnus in Biological Sciences, U. of Delaware, 2010.

### **MEMBERSHIPS IN PROFESSIONAL SOCIETIES**

Poultry Science Association, Inc. (PSA).  
Worlds Poultry Science Association (WPSA).  
American Physiological Society (APS).

### **UNIVERSITY SERVICE (1981-1990)**

Department Committees: 17  
College Committees: 5  
University Committees: 3  
University Faculty Senate 1987-1988 (Alternate Replacement).

### **UNIVERSITY SERVICE (1991-present)**

*College Search Committee for the Position of Head, Department of Poultry Science*  
(Chair, 1991).  
*College Research Peer Review Committee* (1990-1993; Chair, 1991).  
*University Selection Committee* for Early Admission to the University of Pennsylvania  
School of Veterinary Medicine (1987-1993).  
*Intercollege Physiology Program Curriculum Committee* (Chair, 1992-1993).  
*Department of Poultry Science P&T Committee* (Chair, 1992-1993).  
*Committee for Consolidating the Animal Sciences Curricula* (Chair, 1993).

*Course Coordinator for Physiology 571 (1992-1993).*  
*College Advisory Committee to the University Veterinarian (1992).*  
*Poultry Science Course and Curriculum Review Committee (1992-1993).*  
*Poultry Science Long Range Planning Committee (1992-1993).*  
*Poultry Science Biological and Chemical Hazards Committee (Chair, 1993).*  
*Department of Poultry Science Poultry Care Research Facility Committee (1994)*  
*Poultry Environmental Research Laboratory (Director, 1994-present)*  
*Department of Poultry Science Graduate Student Screening Committee (1994-present)*  
*Department of Poultry Science Space/Facilities Coordinator (1994-1995)*  
*College Senior Faculty Committee (1994-present)*  
*College Curriculum Committee (1994-1997)*  
*Chair, POSC Search Committee for Undergraduate Curriculum Coordinator (1994-1997)*  
*Environmental Task Force, University of Arkansas (1995)*  
*Farm Operations Task Force, College of Agriculture (1995)*  
*Poultry Science Search Committee for Extension Group Leader (1997)*  
*Poultry Science Tenure and Promotion Committee (1997-present)*  
*Poultry Science Graduate Admissions Committee (1996-present)*  
*Biological Sciences Search Committee for Physiologist (1998)*  
*Poultry Science Search Committee for Physiologist (1999)*  
*Poultry Science Ph.D. Program Assessment Committee for ADHE (2001)*  
*College Search Committee for Associate Dean of the Experiment Station (2002)*  
*Poultry Science Dept Farm Policy Committee (2002)*  
*Poultry Science Dept Farm Energy & Utilities Committee (2002)*  
*College Spitze Land Grant Award Committee (2003)*  
*College Animal Biotechnology Committee (2003-2004)*  
*College Research Initiation Fund Proposal Evaluation Committee (2003 - 2006).*  
*Faculty Co-Advisor to the Poultry Science Graduate Association (2003 - 2007).*  
*Curriculum Coordinator, Food Safety and Quality Program (2004 - 2005).*  
*CEMB Faculty Member (2004-present).*  
*Cohort Leader, New Graduate Student Orientation (2004 - 2008).*  
*DBCAFLS Multi-County Strategic Planning Facilitator (2004).*  
*DBCAFLS Centennial Symposium Committee (2005).*  
*Faculty Co-Advisor to the Poultry Science Club (undergraduate) (2004 - 2007).*  
*Poultry Science Search Committee (Chair) for Genomics/Proteomics Faculty (2006)*  
*Director - DNA Resource Center, University of Arkansas (2006 – present), see:*  
[http://www.poultryscience.uark.edu/DNA\\_center/dna\\_web.html](http://www.poultryscience.uark.edu/DNA_center/dna_web.html).

## **PROFESSIONAL SERVICE**

*Poultry Science: Associate Editor for the Physiology and Reproduction Section (1985-1988; 1996-present). Section Editor for the Physiology, Endocrinology and Reproduction Section (2001-2008).*  
*Journal of Applied Poultry Research: Editorial/Review Board (1992-present).*  
*Poultry Science Association: Graduate Student Manuscript Committee (1992-1994);*  
*Chair, Environment-Management Session, Environmental Microbiology (1997); PSA*  
*Board of Directors (1997-2000); Chair, PSA Poster Sessions (1998); PSA*  
*Constitution Committee (1998-2000); PSA Board Liaison to: Association for the*  
*Assessment and Accreditation of Laboratory Animal Care International (AAALAC)*  
*(1998), Purina Mills Fellowship Committee (1998), PSA Committee on Animal Care*  
*(1998), PSA Student Research Manuscript Award Committee (1999), Poultry*  
*Science Association, Inc. Research Award Committee (1999), PSA Careers*

Committee (1999), Americal Registry of Professional Animal Scientists (1999), Association of Executive Officers in Animal Sciences (1999), Extension Committee (2000), FASS Committee (1999-2003). PSA Graduate Student Presentation Judge (2004).

*CSRS Review Team*: University of Georgia Department of Poultry Science (1993).

*Federation of Animal Science Societies (FASS)*:

Board of Directors (1998-2001), President-Elect (1999-2000)

President (2000-2001): Policies and Procedures Manual written and implemented; Performance Evaluation Protocols established for Executive Vice Presidents.

Past-President (2001-2002): Strategic Planning Co-Coordinator (2002).

*USDA National Task Force on Research, Education, and Economics* (2003-2004).

Appointed by Secretary of Agriculture Ann Veneman. Co-author of the Task Force report: "National Institute for Food and Agriculture, a Proposal", July, 2004.

*National Coalition for Agricultural Research (N-CFAR)* (2004–2005): Elected to represent the Federation of Animal Science Societies on the N-CFAR Board. Appointed Chair of

the Membership Committee 2004, 2005.

*International Conference on Avian Nutritional and Metabolic Disorders* (Co- Organizer with Professor Xiaolong Wang: <http://anmd.njau.edu.cn/> 2005-2006)

*Occasional Manuscript Reviews* for the following journals:

Americal Journal of Physiology

Endocrinology

General and Comparative Endocrinology

Journal of Applied Physiology

Journal of Comparative Physiology

Avian Pathology

British Poultry Science

Veterinari Medicina - Czech

## **SABBATICAL LEAVE**

Collaborative research with Dr. W. G. Bottje and Dr. R. P. Glahn (Spring, 1990; Department of Animal Science, University of Arkansas, Fayetteville, AR).

## **TEACHING EXPERIENCE**

***University of Connecticut*** (Graduate Teaching Asistantship)

Human Anatomy and Physiology Laboratory

1974-1977

Microtechnique Laboratory

1977-1978

***University of Arizona College of Medicine***

Medical Physiology Laboratory

1978-1981

Physiology for Biomedical Engineers

1979-1981

***The Pennsylvania State University***

Physiology 572 (Graduate Endocrinology)

1981-1988

Avian Physiology 597a, 497a

1981-1987

Physiology 571 (Renal Physiology)

1988-1993

Comparative Physiology of Domestic Animals 407

1989-1993

Poultry Science 495 Internship Coordinator

1991-1993

### **Undergraduate Advising**

15 to 20 students/semester in the Animal Bioscience Program 1981-1991  
6 students/semester in Poultry Technology & Management 1991-1993

### **Student and Peer Evaluations of Teaching**

Consistently ranked in the highest categories by student and peer evaluators in PHSIO 571 and PTYSC/PHSIO 407 (means for overall course evaluations range from 6.3 to 6.5 on a scale where 7 is the highest rating and 1 is the lowest rating).

**University Scholars Honors Thesis Research Director:** 7 students (1983-1993).

### **Undergraduate Authorships or Co-Authorships on Refereed Journal Publications:**

Klinefelter, B.B. (1984); J.G. Unflat (1985); R.E Kissell (1985); R.A. Niznik (1985); W.J. Mitsos (1987); K.R. Bennett (1987); S.P. Smith (1987); T. Reilly (1987); J.W. Evangelisti (1988); R.S. Shapiro (1989); T.H. Lac (1990); H.L. Anthony (1990); M.R. Sima (1990); L. Holt (1992); J.S. Pla (1992); A.C. Nissley (1992).

### ***The University of Arkansas***

POSC/ANSC 5933 (Graduate Environmental Physiology)	1993-1996
VTSC 3033 (Veterinary Physiology; 7 lectures taught)	1993-1995
POSC 5022 (Special Problems; Ascites)	1993-1999
POSC 5243 (Avian Physiology)	1995-1999
POSC/ANSC 3042 (Animal/Poultry Physiology II)	1995-2009
POSC 2554 (Poultry Biology; 3 lectures 2 labs)	1996-1999
POSC 5954 (Digestive, Renal and Respiratory Physiology)	1998-1999
POSC/ANSC 5972 (Renal Physiology)	2000-present
POSC/ANSC 5952 (Respiratory Physiology)	2010-present

### **Student and Peer Evaluations of Teaching**

Consistently ranked in the highest categories by students in POSC/ANSC 3042 (4.8 out of 5), and POSC/ANSC 5972 (4.5 out of 5).

### **Undergraduate Authorships or Co-Authorships on Refereed Journal Publications:**

C.J. Tressler (1996); N.E. Marson, (1996, 1998, 1999); C.D. Tackett (1996, 1998); S. Stebel (2008).

**Honors Thesis Research Director:** Michael T. Bayona. The Distribution of Complex Vascular Lesions in Broiler Chickens with Idiopathic Pulmonary Arterial Hypertension. Honors Thesis in Biology (2009).

### **Web-CT Course Development:**

All lecture notes and handouts, review summaries, example exams, and powerpoint figures for the POSC/ANSC 3042 Animal Physiology II course were installed by Dr. Wideman on the University of Arkansas Web-CT site. This Web-CT site is updated annually and is used during Spring semester, 2000-present.

**Food Safety and Quality Programs for Distance Education:**

Assisted in planning, organizing, and implementing the FS&Q distance education programs for a non-thesis Master's Degree and for two Undergraduate Certificates. Ongoing service as Program Advisor for the HACCP Coordinator Certificate for undergraduate credit, and for the Food Safety Manager Certificate for Undergraduate credit (<http://www.uacted.uark.edu/>).

**GRADUATE STUDENT PROGRAMS DIRECTED OR CO-DIRECTED**

Jay Koch (Thesis Co-Directed with Dr. E. G. Buss)

*Thesis:* The Ability of Laying and Non-Laying Chickens from Thick- and Thin- Shell Lines to Mobilize Calcium in Response to Hypocalcemia Created by EGTA Injection. M.S. in Genetics, 1982.

Linda L. Hnatow

*Thesis:* The Effects of the Mycotoxin Citrinin on Kidney Function of SCWL Pullets. M.S. in Poultry Science, 1984.

Jo A. Closser

*Thesis:* The Effects of Diet and Infectious Bronchitis on Urolithiasis in Poultry. M.S. in Poultry Science, 1985.

Raymond P. Glahn

*Thesis:* The Dose Response Effects of the Mycotoxin Citrinin on Kidney Function of SCWL Pullets. M.S. in Physiology, 1986.

*Thesis:* The Causes and Treatment of Urolithiasis in Single Comb White Leghorn Domestic Fowl. Ph.D. in Physiology, 1989.

*Award:* Graduate Student Research Paper Certificate of Excellence, Poultry Science Association 1987 Annual Meeting, Corvallis, OR.

*Award:* Graduate Student Research Paper Certificate of Excellence, Poultry Science Association 1988 Annual Meeting, Baton Rouge, LA.

*Award:* Young Investigator Award for Excellence in Renal Physiology. American Physiological Society 1992 Annual Meeting, Anaheim, CA.

Timothy S. Stanton

*Thesis:* The Effects of Dietary Available Phosphorus and Fat Source on the Phosphaturic Response to Parathyroid Hormone. M.S. in Physiology, 1987.

*Award:* Graduate Student Research Paper Certificate of Excellence, Poultry Science Association 1986 Annual Meeting, Raleigh, NC.

Vincent E. Vena

*Thesis:* Effect of Dietary Sodium on Glomerular Filtration Rate Autoregulation in Single Comb White Leghorn Domestic Fowl. M.S. in Physiology, 1989.

*Award:* First Place, Life and Health Sciences Category, 1988 Penn State University Graduate Student Research Competition.

Amy J. Lent

*Thesis:* Comparisons of Alimet, D,L-Methionine and Ammonium Sulfate as Dietary Acidifiers for Treating Urolithiasis in Domestic Fowl.

M.S. in Physiology, 1990.

Robert L. Owen, VMD (Thesis Co-Directed with Dr. Barrett S. Cowen)  
*Thesis:* The Pathophysiology of Broiler Pulmonary Hypertension Syndrome.  
Ph.D. in Veterinary Science, 1992.  
*Award:* Third Place, 1989 P.S.U. Graduate Student Research Competition.

Mary F. Forman  
*Thesis:* The Renal Contribution to Pulmonary Hypertension Syndrome in Broilers.  
Ph.D. in Poultry Science, 2000.

Ciro Abel Ruiz-Feria (Mexican Congressional Fellowship)  
*Thesis:* The Role of L-Arginine and Taurine in the Pathophysiology of Pulmonary Hypertension Syndrome (PHS, Ascites) in Broiler Chickens.  
Ph.D. in Poultry Science, 2001.  
*Award:* Graduate Student Research Paper Certificate of Excellence in Physiology, Poultry Science Association 2000 Annual Meeting, Montreal, Canada.  
*Award:* Graduate Student Manuscript Award, Poultry Science Association 2001 Annual Meeting, Indianapolis, IN.

Wenjun Wang (Thesis Co-Directed with Dr. Gisela F. Erf)  
*Thesis:* Cardio-Pulmonary Hemodynamic and Immunological Responses to Endotoxin in Broilers Under Various Environmental Conditions.  
Ph.D. in Poultry Science, 2003.

Mark Chapman  
*Thesis:* Role of Serotonin in Broiler Pulmonary Vasoconstriction  
M.S. in Poultry Science, 2003.  
*Award:* Graduate Student Research Paper Certificate of Excellence in Physiology, Poultry Science Association 2004 Annual Meeting, St. Louis, MO.  
*Thesis:* Evaluation of the Role of Serotonin in Pulmonary Arterial Hypertension in Broilers Induced by Bacterial Lipopolysaccharide and Cellulose Microparticles  
Ph.D. in Poultry Science, 2007.

Olivia T. Bowen (Co-Advised with Dr. Gisela F. Erf)  
*Thesis:* Influence of Nitric Oxide on Broiler's Pulmonary Hypertensive Response to Lipopolysaccharide using Nitric Oxide Synthase Inhibitors.  
M.S. in Poultry Science, 2005.  
*Award:* Graduate Student Research Paper Certificate of Excellence, Poultry Science Association 2006 Annual Meeting, Edmonton, Canada.  
Ph.D. in Poultry Science 2008.

Krishna Hamal (Co-Advised with Dr. Gisela F. Erf)  
*Thesis:* Gene Expression in the Lungs of Ascites-Susceptible and-Resistant Broilers Undergoing Inflammatory responses to Entrapped Microparticles.  
Ph.D. in Poultry Science, 2008.

Alberto Gino Lorenzoni Calvo  
*Thesis:* Cardio-Pulmonary Characteristics of Pulmonary Hypertension in Broilers Including the Impact of Aerosolized Lipopolysaccharide.

Ph.D. in Poultry Science 2008.

Kendall Nicole Mitchell (MS in preparation)  
John Matthew Stark (MS in preparation)

## **MEMBERSHIP ON GRADUATE STUDENT COMMITTEES**

George Boggan (U. of Pennsylvania Vet. School)  
Suzanne Bachman (M.S. Physiology)  
Mark Rovito (M.S. Physiology)  
Bruce Freedman (M.S. Nutrition)  
Sandra L. Youtz (M.S. Nutrition)  
Jeanne Bachman (M.S. Physiology)  
Elizabeth Crandall (Ph.D. Biology)  
Charles Perrotta (M.S. Vet. Science)  
Eid Haddad (Ph.D. Physiology)  
Gerald P. Ballough (Ph.D. Physiology)  
Beverly L. Roeder, D.V.M. (Ph.D. Vet. Science)  
James Trout (Ph.D. Physiology)  
Kathleen Melanson (M.S. Nutrition)  
Tsao Po-Hung (M.S. Animal Science)  
Robert L. Owen, V.M.D. (M.S. Vet. Science)  
Claudia P. Alfonso (Ph.D. Vet. Science)  
Dion Zappe (Ph.D. Physiology)  
Gilbert L. Hendricks (Ph.D. Physiology)  
Jayanti K. Dash (Ph.D. Poultry Science)  
Sui-Ying Wang (Ph.D. Poultry Science)  
Abraheem Al-Arifi (Ph.D. Agricultural Engineering)  
Z. Song (Ph.D. in Poultry Science)  
Jay Hughes (M.S. in Poultry Science)  
David Cawthon (Ph.D. in Poultry Science)  
Babu Sudharshan (M.S. in Agricultural Engineering)  
Thilakar Rathinam (M.S. in Poultry Science)  
Murali K. Devabhaktuni (M.S. in Poultry Science)  
Philip M. Maynard (Ph.D. in Poultry Science)  
Carolyn Ojano-Dirain (Ph.D. in Poultry Science)  
Padmakumar B. Pillai (Ph.D. in Poultry Science)  
Suda Kakarla (M.S. in Poultry Science)  
Amruta Shriram-Kotbagi (M.S. in Human and Environmental Science)  
Kentu Lassiter (M.S. in Cell & Molecular Biology)  
Farrah N. Madison (Ph.D. in Poultry Science)  
Joseph Agugliaro (Ph.D. in preparation, Biological Sciences)  
Komal Singh Rasaputra (Ph.D. in preparation, Poultry Science)  
Michael Gregory (MS in Agricultural, Food & Life Sciences – Food Safety)  
Adnan Al-Rubaye (Ph.D. in preparation, Cell & Molecular Biology)  
Grant Mason (MS in preparation, Poultry Science)  
Susan Stebel (MS in preparation, Animal Science)  
Sriram Krishnamoorthy (Ph.D. in preparation, Poultry Science)  
Jesse A. Stafford (MS in preparation, Kinesiology)

## RESEARCH FUNDING

### *Extramural Grants and Projects Funded (1997-present)*

Title: Pathophysiology of Pulmonary Hypertension Syndrome in Poultry  
Funding Agency: Hubbard ISA  
Period of Support: 1997  
Total Dollar Amounts: \$17,500  
R.F. Wideman, P.I.  
Status: Funded

Title: An Integrated Approach to Evaluating Inherited Predictors of Resistance to Pulmonary Hypertension Syndrome (Ascites) in Fast Growing Broiler Chickens.  
Funding Agency: BARD/USDA (Binational Agricultural Research and Development Fund)  
Period of Support: 1997-2000  
Total Dollar Amounts: \$380,000  
R.F. Wideman, P.I. (Anthony, Cahaner, Roush, Shlosberg, Co-I)  
Status: Funded

Title: Pathophysiology of Pulmonary Hypertension Syndrome in Poultry  
Funding Agency: Hubbard ISA  
Period of Support: 1998  
Total Dollar Amounts: \$15,500  
R.F. Wideman, P.I.  
Status: Funded

Title: L-Carnitine and Ascites in Broilers  
Funding Agency: Rhone-Poulenc and the Lonza Co.  
Period of Support: 1999  
Total Dollar Amounts: \$10,000  
R.F. Wideman, P.I.  
Status: Funded

Title: Pathophysiology of Pulmonary Hypertension Syndrome in Poultry  
Funding Agency: Hubbard ISA  
Period of Support: 2000  
Total Dollar Amounts: \$17,500  
R.F. Wideman, P.I.  
Status: Funded

Title: Mitochondrial Dysfunction in Pulmonary Hypertension Syndrome in Broilers  
Funding Agency: USDA  
Period of Support: 1999-2002  
Total Dollar Amounts: \$200,000  
W.G. Bottje, P.I. (R.F. Wideman, Co-PI, 5% effort).  
Status: Funded

Title: Pathophysiology of Pulmonary Hypertension Syndrome in Poultry  
Funding Agency: Hubbard ISA

Period of Support: 2001-2002  
Total Dollar Amounts: \$18,000  
R.F. Wideman, P.I.  
Status: Funded

Title: Evaluation of Sodium Bicarbonate as a Treatment for Reducing the Incidence of Ascites in Broilers Exposed to Cool Environmental Temperatures  
Funding Agency: Church & Dwight Co., Inc.  
Period of Support: 2000-2001  
Total Dollar Amounts: \$5,000  
R.F. Wideman, P.I.  
Status: Funded

Title: Pathophysiology of Pulmonary Hypertension Syndrome in Poultry  
Funding Agency: Hubbard ISA  
Period of Support: 2002-2003  
Total Dollar Amounts: \$18,500  
R.F. Wideman, P.I.  
Status: Funded

Title: A Practical Method for Inducing Molting of Caged Layers that Combines Full Access to Feed and Water, Dietary Thyroactive Protein, and Short Day Length.  
Funding Agency: United Egg Producers  
Period of Support: 2003-2004  
Total Dollar Amounts: \$20,000  
R.F. Wideman, P.I., W.J. Kuenzel and D.M. Hooge, Co-PI  
Status: Funded

Title: Intravenous Micro-Particle Injections and Pulmonary Hypertension Syndrome in Broilers  
Funding Agency: USDA  
Period of Support: 2003-2006  
Total Dollar Amounts: \$183,999  
R.F. Wideman, P.I., G.F. Erf, Co-P.I.  
Status: Funded.

Title: Response of Two Genetic Lines to a Cyclic Heat Challenge  
Funding Agency: Hubbard  
Period of Support: 2004  
Total Dollar Amounts: \$27,279  
R.F. Wideman, G.F. Erf, Co-P.I.  
Status: Funded

Title: Broiler Breeder Molting Trial with Dietary Thyroid Hormones  
Funding Agency: Sub-Contract from Danny M. Hooge's USDA-SBIR Grant  
Period of Support: 2005  
Total Dollar Amounts: \$15,000  
R.F. Wideman, K. Bramwell, Co-P.I.  
Status: Funded

Title: Validation of an Animal Model for Human Pulmonary Arterial Hypertension  
Funding Agency: Arkansas Biotechnology Institute (ABI)  
Period of Support: 2007-2008  
Total Dollar Amounts: \$66,310  
D. Rhoads, P.I., N.B. Anthony, G.F. Erf, R.F. Wideman, Co-P.I.  
Status: Funded

Title: Red Blood Cell Function in Nitric Oxide Biotransport  
Funding Agency: NIH  
Period of Support: 2007-2008  
Total Dollar Amounts: \$150,000  
M. Kavdia, P.I., R.F. Wideman, Co-P.I. (5% effort)  
Status: Funded

Title: Pulmonary Arterial Hypertension: Avian Model of Complex Vascular Lesion Development.  
Funding Agency: NIH  
Period of Support: 2008-2011  
Total Dollar Amounts: \$209,283  
R.F. Wideman, P.I., N.B. Anthony, Co-P.I., G.F. Erf, Co-P.I., D.D. Rhoads, Co-P.I.  
Status: Funded

Title: Validation of an Animal Model for Human Pulmonary Arterial Hypertension.  
Funding Agency: Arkansas Biosciences Institute  
Period of Support: 07/08-06/09  
Total Dollar Amounts: \$50,000  
N.B. Anthony, P.I., G.F. Erf, Co-P.I., D.D. Rhoads, Co-P.I., R.F. Wideman, Co-PI..  
Status: Funded

Title: Gene Expression and Candidate Gene Analysis in an Animal Model for Human Pulmonary Arterial Hypertension.  
Funding Agency: Arkansas Biosciences Institute  
Period of Support: 11/08-10/10  
Total Dollar Amounts: \$103,925  
D.D. Rhoads, P.I., G.F. Erf, Co-P.I., N.B. Anthony, Co-P.I., R.F. Wideman, Co-PI.  
Status: Funded

Title: Developing a Research Model for Exposing Susceptibility to Leg Problems.  
Funding Agency: Cobb-Vantress  
Period of Support: 02/09-05/09  
Total Dollar Amounts: \$8,645  
R.F. Wideman, PI.  
Status: Funded

Title: Developing a Research Model for Exposing Susceptibility to Leg Problems.  
Funding Agency: Cobb-Vantress  
Period of Support: 06/09-09/09  
Total Dollar Amounts: \$12,649  
R.F. Wideman, PI.  
Status: Funded

Title: Fine-Tuning a Research Model for Exposing Susceptibility to Lameness.  
Funding Agency: Cobb-Vantress  
Period of Support: 10/09-09/10  
Total Dollar Amounts: \$75,216  
R.F. Wideman, PI.  
Status: Funded

Title: Contribution of Litter to Body Weight Gain in Broilers Grown on Wire Flooring.  
Agency: Biomin Holding GmbH  
Years Funded: 12/09-02/10  
Total Amount: \$3,369  
Annual Amount: \$3,369  
My Share This Year: 100%; R.F. Wideman, PI; no Co-I.  
Status: Funded

Title: Efficacy of Biomin Probiotic for Reducing the Incidence of Lameness in Broilers Grown on Wire Flooring.  
Agency: Biomin Holding GmbH  
Years Funded: 3/10-12/10  
Total Amount: \$4,369  
Annual Amount: \$4,369  
My Share This Year: 100%; R.F. Wideman, PI; no Co-I.  
Status: Funded

Title: Sudden Death Syndrome (SDS) Pilot Study: Initial Comparison of SDS-Susceptible Line versus Base Line.  
Funding Agency: Cobb-Vantress  
Period of Support: 05/10-07/10  
Total Dollar Amounts: \$6,517  
R.F. Wideman, PI.  
Status: Funded

Title: Vascular Mechanisms Associated with the Development of Plexiform lesions in Chickens with Idiopathic Pulmonary Arterial Hypertension.  
Funding Agency: Arkansas Biosciences Institute  
Period of Support: 07/10-07/11  
Total Amount: \$28,839  
Annual Amount: \$28,838  
My Share This Year: 5%; H. A. Kluess, P.I., R.F. Wideman, Co-PI.  
Status: Funded

Title: Efficacy of Biomin Probiotic for Reducing the Incidence of Lameness in Broilers Grown on Wire Flooring.  
Agency: Biomin Holding GmbH  
Years Funded: 9/8/10-3/7/2011  
Total Amount: \$14,574  
Annual Amount: \$14,574  
My Share This Year: 100%; R.F. Wideman, PI; no Co-I.  
Status: Funded

### ***Internal Funding***

Department Hatch Funds pay the salaries and benefits of Dr. Wideman and for one Program Associate I. One state-funded graduate assistantship is provided. State funds of \$3,000 are provided for hourly wages, mailing and copying costs, travel, and general supplies.

Title: The Role of the Immune System in the Development of Pulmonary Hypertension Syndrome in Broilers

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 1999

Total Dollar Amounts: \$10,582

G. F. Erf P.I., R.F. Wideman, Co-I.

Status: Funded

Title: The Role of the Immune System in the Development of Pulmonary Hypertension Syndrome in Broiler Chickens

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2000

Total Dollar Amounts: \$15,000.

G. F. Erf P.I., R.F. Wideman, Co-I.

Status: Funded

Title: The Role of the Immune System in the Development of Pulmonary Hypertension Syndrome in Broiler Chickens

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2001

Total Dollar Amounts: \$15,000.

G. F. Erf P.I., R.F. Wideman, Co-I.

Status: Funded

Title: Exertional Myopathy in Turkeys: Pathophysiology and Impact on Meat Quality.

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2002

Total Dollar Amounts: \$13,800

C. M. Owens, P.I., R.F. Wideman, H. D. Chapman, F. D. Clark, S. Watkins, Co-PI

Status: Funded

Title: Impact of the Intravenous Micro-Particle Selection Technique on the Pulmonary Hypertensive Response of Broilers to LPS.

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2003

Total Dollar Amounts: \$12,000

R.F. Wideman, P.I., G. F. Erf, Co-I

Status: Funded

Title: The Role of Serotonin (5-HT) in Ascites (PHS) in Broilers: The *in vivo* Release and Subsequent Pulmonary Vascular Response to 5-HT During the Inflammatory Response Initiated by Entrapped Micro-Particles, and Evaluation of the Impact of bacterial Lipopolysaccharide (LPS) on 5-HT Release.

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2004

Total Dollar Amounts: \$12,000

M. E. Chapman and R.F. Wideman, Co-P.I.

Status: Funded

Title: The Role of Serotonin (5-HT) in Ascites (PHS) in Broilers: The *in vivo* Release and Subsequent Pulmonary Vascular Response to 5-HT During the Inflammatory Response Initiated by Entrapped Micro-Particles, and Evaluation of the Impact of bacterial Lipopolysaccharide (LPS) on 5-HT Release.

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2005

Total Dollar Amounts: \$11,500

M. E. Chapman and R.F. Wideman, Co-P.I.

Status: Funded

Title: The Role of Serotonin (5-HT) in Ascites (PHS) in Broilers: The *in vivo* Release and Subsequent Pulmonary Vascular Response to 5-HT During the Inflammatory Response Initiated by Entrapped Micro-Particles, and Evaluation of the Impact of bacterial Lipopolysaccharide (LPS) on 5-HT Release.

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2006

Total Dollar Amounts: \$11,500

M. E. Chapman and R.F. Wideman, Co-P.I.

Status: Funded

Title: Respiratory Response of Broilers to Inhalation of Bacterial Lipopolysaccharide (LPS, Endotoxin): Development of an Aerosol Inhalation Model and Characterization of Vasoconstrictors Contributing to the Ensuing Pulmonary Arterial Hypertension

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2006-2007 (year 1 of 3)

Total Dollar Amounts: \$11,340

R.F. Wideman, P.I.

Status: Funded

Title: Respiratory Response of Broilers to Inhalation of Bacterial Lipopolysaccharide (LPS, Endotoxin): Development of an Aerosol Inhalation Model and Characterization of Vasoconstrictors Contributing to the Ensuing Pulmonary Arterial Hypertension

Funding Agency: Univ. of Arkansas Animal Health Proposal

Period of Support: 2007-2008 (year 2 of 3)

Total Dollar Amounts: \$11,500

R.F. Wideman, P.I.

Status: Funded

Title: Respiratory Response of Broilers to Inhalation of Bacterial Lipopolysaccharide (LPS, Endotoxin): Development of an Aerosol Inhalation Model and Characterization of Vasoconstrictors Contributing to the Ensuing Pulmonary Arterial Hypertension  
Funding Agency: Univ. of Arkansas Animal Health Proposal  
Period of Support: 2008-2009 (year 3 of 3)  
Total Dollar Amounts: \$11,500  
R.F. Wideman, P.I.  
Status: Funded

## **PATENTS, COPYRIGHTS AND LICENSES APPLIED FOR OR RECEIVED**

US Patent Number 5,895,788 Use of L-arginine and salts thereof in drinking water for the prevention and/or treatment of pulmonary hypertension syndrome in avians. Patent awarded 1999.

US Patent Number 6,127,421 In Ovo use of L-arginine and salts thereof for the prevention and/or treatment of pulmonary hypertension syndrome in avians. Patent awarded 2000.

US Patent Number 6,720,473 Intra-Vascular administration of particles to induce pulmonary hypertension, pulmonary hypertension syndrome, and ascites in animals. Patent awarded 2004.

US Provisional Patent Applications 60/586,104 Composition and Methods for Improved Avian Performance filed by Danny M. Hooge on July 7, 2005 is based on intellectual property developed by R. F. Wideman et al. (funded as: "A Practical Method for Inducing Molting of Caged Layers that Combines Full Access to Feed and Water, Dietary Thyroactive Protein, and Short Day Length. Funded by United Egg Producers, 2003-2004, \$20,000; R.F. Wideman, P.I., W. J. Kuenzel and D. M. Hooge, Co-PI) and licensed to Hooge Consulting Service, Inc. by the University of Arkansas.

## **PUBLICATIONS (Chronological Listings by Category)**

### ***Doctoral Dissertation***

Wideman, R. F. Parathyroid Ultrastructure and Function in the European Starling, *Sturnus vulgaris*. Ph.D. Thesis. University of Connecticut, Storrs, CT. 228pp., 1978. (Nancy Barnes Clark, Dissertation Director).

### ***Book Chapters and Review Articles***

1. Wideman, R. F. The Physiology of Edema and Ascites in Poultry. Proceedings: VII Ciclo Internacional de Conferencias Sobre Avicultura. Pages 189-217, 1984.
2. Wideman, R. F., Diuresis en Aves. Proceedings: VIII Ciclo de Conferencias Internacionales Sobre Avicultura. Pages 143-168, 1987.

3. Wideman, R. F. Renal regulation of avian calcium and phosphorus metabolism. *J. Nutrition* 117:808-815, 1987.
4. Wideman, R. F. Kidney Anatomy and Physiology. In: *CRC Critical Reviews in Poultry Biology*. Ed. R. R. Dietert. CRC Press, Volume 1 (2):133-176, 1988.
5. Clark, N. B. and Wideman, R. F. Actions of Parathyroid Hormone and Calcitonin in Avian Osmoregulation. In: *Progress in Avian Osmoregulation*. Eds. M. R. Hughes and A. Chadwick. Leeds Philosophical and Literary Society (Scientific Section), Leeds, United Kingdom, Pages 111-125, 1989.
6. Wideman, R. F. Economic aspects of renal dysfunction in poultry. In: *Optimizing Resources in Animal Protein Production*, Proceedings of the 1991 Novus Technical Symposia, Aruba, Netherlands Antilles, and Huatulco, Oaxaca, Mexico. pp65-87, 1993.
7. Wideman, R. F., and Bottje, W. G. Current Understanding of the Ascites Syndrome and Future Research Directions. Proceedings of the Novus International Inc. Nutritional and Technical Symposium, Springdale AR and Atlanta GA. pp1-20, 1993.
8. Bottje, W. G., and Wideman, R. F. Potential Role of Free Radicals in the Pathogenesis of Pulmonary Hypertension Syndrome. In: *Poultry and Avian Biology Reviews*. Ed. R. R. Dietert and M. A. Ottinger. Volume 6(3):211-231, 1995.
9. Wideman, R. F. Cardio-Pulmonary Hemodynamics and Ascites in Broiler Chickens. In: *Poultry and Avian Biology Reviews*. Ed. R. R. Dietert and M. A. Ottinger. Volume 11(1):21-43, 2000.
10. Wideman, R. F. Pathophysiology of Heart/Lung Disorders: Pulmonary Hypertension Syndrome in Broiler Chickens. *World's Poultry Science Journal* 57:289-307, 2001.
11. Wideman, R. F., Chapman, M. E., Hamal, K. R., Bowen, O. T. Lorenzoni, A. G., Erf, G. F., and Anthony, N. B. An inadequate pulmonary vascular capacity and susceptibility to pulmonary arterial hypertension in broilers. *Poult. Sci.* 86:984-998, 2007.
12. Khajali, F., and Wideman, R.F. Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. *World's Poult. Sci. J.* 66: 751-766, 2010, 2010.
13. Wideman, R. F., and K. R. Hamal. Idiopathic pulmonary arterial hypertension: an avian model for plexogenic arteriopathy and serotonergic vasoconstriction. *J. Pharmacol. Toxicol. Methods* (*in press*). 2011

#### ***Refereed Journal Articles***

1. Clark, N. B., Braun, E. J., and Wideman, R. F. The effects of parathyroid hormone on the renal excretion of phosphate and calcium in starlings. *Am. J. Physiol.* 231: 1152-1158, 1976.

2. Clark, N. B., and Wideman, R. F. Renal excretion of phosphate and calcium in parathyroidectomized starlings. *Am. J. Physiol.* 233: F138-F144, 1977.
3. Clark, N. B., and Wideman, R. F. Calcitonin stimulation of urine flow and sodium excretion in the starling. *Am. J. Physiol.* 238: R406-R412, 1980.
4. Wideman, R. F., Clark, N. B., and Braun, E. J. Effects of phosphate loading and parathyroid hormone on renal inorganic phosphate excretion in the starling, *Sturnus vulgaris*. *Am. J. Physiol.* 239: F233-F243, 1980.
5. Wideman, R. F. Parathyroid innervation in the European Starling, *Sturnus vulgaris*. *J. Morphol.* 166: 65-80, 1980.
6. Wideman, R. F., Braun, E. J., and Anderson, G. L. Microanatomy of the domestic fowl renal cortex. *J. Morphol.* 168: 249-267, 1981.
7. Wideman, R. F., and Braun, E. J. Stimulation of avian renal phosphate secretion by parathyroid hormone. *Am. J. Physiol.* 241: F263-F272, 1981.
8. Wideman, R. F., and Braun, E. J. Ureteral urine collection from anesthetized domestic fowl. *Lab. Animal Sci.* 32: 298-301, 1982.
9. Wideman, R. F., Mallinson, E. T., and Rothenbacher, H. Kidney function of pullets and laying hens during outbreaks of urolithiasis. *Poultry Sci.* 62: 1954-1970, 1983.
10. Koch, J., Wideman, R. F., and Buss, E. G. Blood ionic calcium response to hypocalcemia in the chicken induced by Ethyleneglycol-Bis- (b-Aminoethylether)-N,N'-Tetraacetic Acid: Role of parathyroids. *Poultry Sci.* 63: 167-171, 1984.
11. Koch, J., Buss, E. G., and Wideman, R. F. Blood ionic calcium responses of hens from thick-shell and thin-shell lines to Ethyleneglycol-Bis- (b-Aminoethylether)-N,N'-Tetraacetic Acid Injections. *Poultry Sci.* 63: 172-175, 1984.
12. Koch, J., Wideman, R. F., and Buss, E. G. Evaluation of commercial mammalian parathyroid hormone (PTH) radioimmunoassay systems for measuring avian PTH. *Comp. Biochem. Physiol.* 78: 315-317, 1984.
13. Mashaly, M. M., Youtz, S. L., and Wideman, R. F. Hypothyroidism and antibody production in immature male chickens. *Immunol. Pub.* 12: 551-563, 1984.
14. Mallinson, E. T., Rothenbacher, H., Wideman, R. F., Snyder, D. B., Russek, E., Zuckerman, A. I., and Davidson, J. P. Epizootiology, pathology, and microbiology of an outbreak of urolithiasis in chickens. *Avian Dis.* 28: 25-43, 1984.
15. Wideman, R. F. Parathyroid hormone-induced phosphate excretion following preequilibration with  $^{32}\text{P}$ . *Am. J. Physiol.* 246: F373-F378, 1984.
16. Wideman, R. F. Organic phosphate probes of the avian renal phosphate secretory mechanism. *Comp Biochem. Physiol.* 78: 315-319, 1984.

17. Klinefelter, B. B., Youtz, S. L., and Wideman, R. F. Effect of parathyroid hormone on total phosphate and inorganic phosphate in blood, plasma and urine of domestic fowl. *Poultry Sci.* 63: 2285-2291, 1984.
18. Wideman, R. F., and Buss, E. G. Percent shell and plasma mineral concentrations in three strains of domestic fowl selected for thick or thin egg shell production. *Poultry Sci.* 64: 388-395, 1985.
19. Wideman, R. F. and Youtz, S. L. Comparisons of avian renal responses to bovine parathyroid extract, synthetic bovine (1-34) parathyroid hormone, and synthetic human (1-34) parathyroid hormone. *Gen. Comp. Endocrinol.* 57: 480-490, 1985.
20. Laverty, G. L., and Wideman, R. F. Effect of oxidized PTH on blood pressure, plasma minerals, and renal function of domestic fowl. *Gen. Comp. Endocrinol.* 59: 391-398, 1985.
21. Wideman, R. F. and Buss, E. G. Arterial blood gas, pH, and bicarbonate values in laying hens selected for thick- or thin-eggshell production. *Poultry Sci.* 64: 1015-1019, 1985.
22. Unflat, J. G., Kissell, R. E., Wideman, R. F., and Muir, F. V. A comparison of two techniques for determining glomerular size distributions in domestic fowl. *Poultry Sci.* 64: 1210-1215, 1985.
23. Hnatow, L. L., and Wideman, R. F. Kidney function of Single Comb White Leghorn pullets following acute renal portal infusion of the mycotoxin citrinin. *Poultry Sci.* 64: 1553-1561, 1985.
24. Niznik, R. A., Wideman, R. F., Cowen, B. S., and Kissell, R. E. Induction of urolithiasis in Single Comb White Leghorn pullets: effect on glomerular number. *Poultry Sci.* 64: 1430-1437, 1985.
25. Wideman, R. F., J. A. Closser, W. B. Roush, and B. S. Cowen. Urolithiasis in pullets and laying hens: Role of dietary calcium and phosphorus. *Poultry Sci.* 64: 2300-2309, 1985.
26. Kissell, R. E., and Wideman, R. F. Parathyroid transplants and unilateral renal delivery of parathyroid hormone in domestic fowl. *Am. J. Physiol.* 249: R732-R739, 1985.
27. Oldroyd, N. G. and Wideman, R. F. Characterization and composition of uroliths from domestic fowl. *Poultry Sci.* 65: 1090-1094, 1986.
28. Gregg, C. M. and Wideman, R. F. Effects of atriopeptin and chicken heart extract in *Gallus Domesticus*. *Am. J. Physiol.* 251: R543-R551, 1986.
29. Wideman, R. F. and Laverty, G. Kidney function in domestic fowl with chronic occlusion of the ureter and caudal renal vein. *Poultry Sci.* 65: 2148-2155, 1986.

30. Wideman, R. F., Satnick, J. L., Mitsos, W. J., Bennett, K. R. and Smith, S. P. Effect of saline adaptation and renal portal sodium infusion on glomerular size distributions and kidney function in domestic fowl. *Poultry Sci.* 66: 348-356, 1987.
31. Wideman, R. F. and Cowen, B. S. Effect of dietary acidification on kidney damage induced in immature chickens by excess calcium and infectious bronchitis virus. *Poultry Sci.* 66: 626-633, 1987.
32. Cowen, B. S., Wideman, R. F., Rothenbacher, H., and Braune, M. O. An outbreak of urolithiasis on a large commercial egg farm. *Avian Dis.* 31: 392-397, 1987.
33. Glahn, R. P. and Wideman, R. F. Avian diuretic response to renal portal infusions of the mycotoxin citrinin. *Poultry Sci.* 66: 1316-1325, 1987.
34. Glahn, R. P., Mitsos, W. J., and Wideman, R. F. Evaluation of embryonic heart rates as a method for sexing chickens. *Poultry Sci.* 66: 1398-1401, 1987.
35. Reilly, T., Gregg, C. M., Wideman, R. F., and Jarrett-Zaczek, D. A potent hypotensive factor in chicken left ventricle. *Proc. Soc. Exp. Biol. Med.* 186: 288-293, 1987.
36. Cowen, B. S., Wideman, R. F., Braune, M. O. and Owen, R. L. An infectious bronchitis virus isolated from chickens experiencing a urolithiasis outbreak. I. In vivo characterization studies. *Avian Dis.* 31: 878-883, 1987.
37. Wideman, R. F., and Gregg, C. M. A model for evaluating avian renal hemodynamics and glomerular filtration rate autoregulation. *Am. J. Physiol.* 254: R925-R932, 1988.
38. Glahn, R.P., Wideman, R.F., Evangelisti, J.W. and Huff, W.H. Effects of ochratoxin A alone and in combination with citrinin on kidney function of Single Comb White Leghorn pullets. *Poultry Sci.* 67: 1034-1043, 1988.
39. Glahn, R. P., Wideman, R. F., and Cowen, B. S. Effect of Gray strain infectious bronchitis virus and high dietary calcium on renal function of SCWL pullets at 6, 10 and 18 weeks of age. *Poultry Sci.* 67: 1250-1263, 1988.
40. Glahn, R. P., Wideman, R. F., and Cowen, B. S. Effect of dietary acidification and alkalization on urolith formation and renal function in SCWL laying hens. *Poultry Sci.* 67: 1694-1701, 1988.
41. Wideman, R. F., and Satnick, J.L. Physiological evaluation of diuresis in commercial broiler breeders. *British Poultry Sci.* 30: 313-326, 1989.
42. Wideman, R.F., Roush, W.B., Satnick, J.L., Glahn, R.P., and Oldroyd, N.O. Methionine hydroxy analog (free acid) reduces avian kidney damage and urolithiasis initiated by excess dietary calcium. *J. Nutrition* 119: 818-828, 1989.
43. Glahn, R.P., Shapiro, R.S., Vena, V.E., Wideman, R.F., and Huff, W.E. Effects of chronic ochratoxin A and citrinin toxicosis on kidney function of Single Comb White Leghorn pullets. *Poultry Sci.* 68: 1205-1212, 1989.

44. Glahn, R.P., Wideman, R.F., and Cowen, B.S. Order of exposure to high dietary calcium and Gray strain infectious bronchitis virus (IBV) alters renal function and the incidence of urolithiasis. *Poultry Sci.* 68: 1193-1204, 1989.
45. Stanton, T.S., Glahn, R.P., and Wideman, R.F. The effects of dietary phosphorus and parathyroid hormone (PTH) infusion rates on the avian phosphaturic response to PTH. *J. Exp. Biol.* 144: 521-533, 1989.
46. Laverty, G. and Wideman, R. F. Sodium excretion rates and renal responses to acute salt loading in the European Starling. *J. Comp. Physiol. B* 159: 401-408, 1989.
47. Wideman, R. F. Maturation of glomerular size distribution profiles in domestic fowl (*Gallus gallus domesticus*). *J. Morphol.* 201: 205-213, 1989.
48. Gregg, C.M. and Wideman, R.F. Morphological and functional comparisons of normal and hypertrophied kidneys of adult domestic fowl, *Gallus gallus*. *Am. J. Physiol.* 258: F403-F413, 1990.
49. Vena, V.E., Lac, T.H., and Wideman, R.F. Dietary sodium, glomerular filtration rate autoregulation and glomerular size distribution profiles in domestic fowl, *Gallus gallus*. *J. Comp. Physiol. B* 160: 7-16, 1990.
50. Anthony, H.L., Sima, M.R., Satnick, J.L., and Wideman, R.F. Cardiac hypotensive extract (CHE): Responses of SCWL hens fed low- or high-sodium diets, and inhibition of the hypertensive response to arginine vasotocin. *Poultry Sci.* 69: 679-685, 1990.
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#### ***Technical and Industry Press Publications***

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Ruiz-Feria, C. A., Beers, K., Kidd, M. T., and Wideman, R. F. Plasma taurine levels in broilers with pulmonary hypertension syndrome (ascites). *Poultry Sci.* 78 (Supplement 1): 111, 1999.

Forman, M. F., and Wideman, R. F. Dietary furosemide delays ascites mortality in broilers with chronic unilateral pulmonary artery occlusion. *Poultry Sci.* 78 (Supplement 1): 111, 1999.

Wideman, R. F., Maynard, P., and Bottje, W. G. Venous blood pressure in broilers during acute inhalation of 5% carbon dioxide, and during unilateral pulmonary artery occlusion. *Poultry Sci.* 78 (Supplement 1): 31, 1999.

Wideman, R. F., and Tackett, C. D. Cardio-pulmonary function in clinically healthy and preascitic broilers: inhalation of 100% oxygen does not reduce the pulmonary vascular resistance or pulmonary arterial pressure. *Poultry Sci.* 78 (Supplement 1): 31, 1999.

Wideman, R. F., and French, H. Ongoing improvement in the ascites resistance of progeny from the second generation of broiler breeders selected using the chronic unilateral pulmonary artery occlusion technique. *Poultry Sci.* 78 (Supplement 1): 31, 1999.

Wideman, R. F., and Tackett, C. D. Cardio-pulmonary function in broilers reared at warm or cold temperatures: inhalation of 100% oxygen does not reduce pulmonary arterial pressure. *Poultry Sci.* 78 (Supplement 1): 31, 1999.

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Forman, M. F., and Wideman, R. F. The influence of dietary protein and furosemide on renal responses in broilers to systemic hypotension induced by unilateral pulmonary artery occlusion. *Poultry Sci.* 79 (Supplement 1):57, 2000.

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Roush, W. B., Wideman, R. F., Cahaner, A., and Cravener, T. L. Defining minimal growth velocity data needed for artificial neural network detection of pulmonary hypertension syndrome in broilers. *Poultry Sci.* 79 (Supplement 1):113, 2000.

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Virden, W. S., Ward, T. L., Wideman, R. F. Jr., and Kidd, M. T. Cardio-pulmonary evaluations of cold-challenged chicks from parents fed supplemental zinc and manganese amino acid complexes. *Poultry Sci.* 81 (Supplement 1):38, 2002.

Wideman, R. F., and Erf, G. F. Intravenous micro-particle injection and pulmonary hypertension in broiler chickens: Cardio-pulmonary hemodynamic responses. *Poultry Sci.* 81 (Supplement 1):78, 2002.

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post-injection mortality and ascites susceptibility. Poultry Sci. 81 (Supplement 1):78, 2002.

Wideman, R. F., Chapman, M. E., and Wang, W. Pulmonary vascular capacity and resistance to cyclic heat stress in broilers. Poultry Sci. 81 (Supplement 1):81, 2002.

Wideman, R. F., and M. E. Chapman. Immune modulation of the pulmonary hypertensive response to bacterial lipopolysaccharide (LPS, endotoxin) in broilers. Poultry Sci. 82 (Supplement 1):3, 2003.

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Wideman, R. F., and M. E. Chapman. Pulmonary and systemic hemodynamic responses to prostacyclin in broilers. Poultry Sci. 83 (Supplement 1):189, 2004.

Bowen, O. T., R. F. Wideman, and G. F. Erf. Is variation among broilers in their pulmonary hypertensive responsiveness to lipopolysaccharide (LPS) attributable to innate variation in nitric oxide (NO) production by mononuclear cells? Poultry Sci. 84 (Supplement 1):28, 2005.

Chapman, M. E., and R. F. Wideman. Nitric oxide measurements in avian blood: Evaluation of two commonly used assays and the impact of aminoguanidine on plasma nitric oxide after injection of lipopolysaccharide. Poultry Sci. 84 (Supplement 1):32, 2005.

Wideman, R. F., G. F. Erf, and M. E. Chapman. N-Nitro-L-Arginine methyl Ester (L-NAME) amplifies the pulmonary hypertensive response to micro-particle injections in broilers. Poultry Sci. 84 (Supplement 1):55, 2005.

Wideman, R. F., W. J. Kuenzel, M. E. Chapman, C. Golden, and D. M. Hooge. A practical method for inducing molting of caged layers that combines full access to feed and water, dietary L-thyroxine or thyroactive protein, and short day length. Poultry Sci. 84 (Supplement 1):131, 2005.

Wideman, R. F. Improving the pulmonary vascular capacity reduces the susceptibility of broilers to ascites and heat stress. XXX Convencion Annual de ANECA, 27 April, Puerto Vallarta, Jalisco, Mexico (Invited Presentation), 2005.

Wideman, R. F. Kidney damage in laying hens (Urolithiasis). International Conference on Avian Nutritional and Metabolic Disorders (Proceedings pp1-13), 15 April, Nanjing Agricultural University, Nanjing China (Invited Presentation), 2006.

Chapman, M. E., and R. F. Wideman. Evaluation of the serotonin receptor blockers ketanserin and methiothepin on the pulmonary hypertensive responses of broilers to intravenously infused serotonin. Poultry Sci. 85 (Supplement 1):41, 2006.

Chapman, M. E., and R. F. Wideman. Evaluation of the serotonin receptor blocker methiothepin in broilers injected intravenously with lipopolysaccharide and microparticles. *Poultry Sci.* 85 (Supplement 1):41, 2006.

Bowen, O. T., G. F. Erf, M. E. Chapman, and R. F. Wideman Jr. Plasma nitric oxide concentrations in broilers after i.v. injections of lipopolysaccharide or microparticles. *Poultry Sci.* 85 (Supplement 1):48, 2006.

Hamal, K. R., R. F. Wideman Jr., B. L. Plumlee, and G. F. Erf. Time-course of expression of inducible nitric oxide synthase in lungs following intravenous cellulose microparticle injection in three broiler lines. *Poultry Sci.* 85 (Supplement 1):48, 2006.

Wideman, R. F. Inadequate pulmonary vascular capacity and susceptibility to pulmonary hypertension syndrome in broilers. *Poultry Sci.* 85 (Supplement 1):142, 2006.

Bowen, O. T., R. F. Wideman, and G. F. Erf. Altered monocyte/macrophage numbers in blood and organs of chickens injected i.v. with LPS. *Poult. Sci.* 86 (Supplement 1):51, 2007.

Chapman, M. E., R. L. Taylor, and R. F. Wideman. Analysis of plasma serotonin levels and hemodynamic responses following chronic serotonin infusion in broilers challenged with bacterial lipopolysaccharide and microparticles. *Poult. Sci.* 86 (Supplement 1):221, 2007.

Lorenzoni, A. G., R. F. Wideman, and N. B. Anthony. Transpulmonary pressure gradient verifies pulmonary hypertension is initiated by increased arterial resistance in broilers. *Poult. Sci.* 86 (Supplement 1):222, 2007.

Stebel, S., and R. F. Wideman. Pulmonary hemodynamic responses to intravenous prostaglandin E<sub>2</sub> in broiler chickens. *Poult. Sci.* 86 (Supplement 1):535, 2007.

Hamal, K. R., R. F. Wideman, N. B. Anthony, and G. F. Erf. Expression of inflammatory mediators in lungs of ascites-resistant and susceptible broilers in response to microparticles entrapped in the pulmonary vasculature. *Poult. Sci.* 87 (Supplement 1):19, 2008.

Lorenzoni, A. G., and R. Wideman. Intratracheal administration of bacterial lipopolysaccharide elicits pulmonary hypertension in broilers with primed airways. *Poult. Sci.* 87 (Supplement 1):83, 2008.

Taylor, R. L., M. E. Chapman, R. F. Wideman, jr., N. B. Anthony, and C. M. Ashwell. Ascites-resistant and susceptible broiler lines express different genes in their right ventricles. *Poult. Sci.* 88 (Supplement 1):51, 2009.

Hamal, K. R., R. F. Wideman, D. Cross, D. Rhoads, N. Anthony, and G. Erf. Immunohistochemical examination of complex vascular lesions in the lungs of domestic fowl selected for susceptibility to pulmonary arterial hypertension. *Experimental Biology* 154:572, 2009.

Wideman, R. F., K. Hamal, M. Bayona, A. Lorenzoni, D. Cross, D. Rhoads, G. Erf, and N. Anthony. Complex vascular lesions in the lungs of domestic fowl selected for susceptibility to pulmonary arterial hypertension: incidence and histology. *Experimental Biology* 154:572, 2009.

Hamal, K. R., R. F. Wideman, D. Rhoads, N. B. Anthony, and G. F. Erf. Histological and Immunohistochemical Examination of Complex Vascular Lesions in the Lungs of Domestic Fowl Selected for Susceptibility to Pulmonary Arterial Hypertension. Pittsburgh International Lung conference, October 9-10, 2009, Pittsburgh, PA

Wideman, R. F., F. Khajali, K. R. Hamal, A. F. Wideman, and H. Lester. A research model for inducing leg problems in broilers. Poultry Science Association Annual Meeting (Denver, CO), 2010.

Bowen, O. T., R. F. Wideman, R. L. Dienglewicz, and G. F. Erf. Effects of repeated intravenous lipopolysaccharide injection on hematological characteristics of chicken blood. Poultry Science Association Annual Meeting (Denver, CO), 2010.

Krishnamoorthy, S., N. Anthony, D. Rhoads, R. Wideman, and G. Erf. Analysis of ascites susceptibility using genetic markers in commercial broilers. Poultry Science Association Annual Meeting (Denver, CO), 2010.

Al-Rubaye, A. A., N. B. Anthony, G. F. Erf, R. F. Wideman, and D. D. Rhoads. Using quantitative PCR to investigate three candidate genes related to pulmonary hypertension in the chicken. Poultry Science Association Annual Meeting (Denver, CO), 2010.

Wideman, R. F., M. L. Eanes, K. R. Hamal, R. Klintworth, and N. B. Anthony. Pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome: Age and gender comparisons. Poultry Science Association Annual Meeting (Denver, CO), 2010.

Wideman, R. F., K. R. Hamal, F. Khajali, A. F. Wideman, and H. Lester. A research model for inducing leg problems in broilers. Biomin World Nutrition Forum (Salzburg, Austria) October 13-16, 2010.

#### **SEMINARS AND TECHNICAL SPEAKING ENGAGEMENTS** (Chronological Listing)

Wideman, R. F. Avian tubular phosphate secretion. The University of Arizona College of Medicine, Tucson, AZ (Invited Presentation) 1979.

Wideman, R. F. Avian renal responses to parathyroid hormone. The Pennsylvania State University Department of Poultry Science (Invited Presentation), 1980.

Wideman, R. F. Micro-anatomy of the domestic fowl kidney. The Pennsylvania State University Department of Poultry Science Seminar, 1982.

Wideman, R. F. The physiology of avian renal phosphate secretion. The Pennsylvania State University Department of Poultry Science Seminar, 1983.

Wideman, R. F. Renal inorganic phosphate secretion. The Pennsylvania State University Biological Research Society (Invited Presentation), 1983.

Wideman, R. F. Urolithiasis and visceral gout in caged layers. Poultry Science Sales and Service Conference, The Pennsylvania State University, 1983.

Wideman, R. F. Model system for renal phosphate secretion. Joint Physiology Program, Milton S. Hershey Medical Center, Hershey, PA, 1984.

Wideman, R. F. Urolithiasis: Kidney stones in poultry. The Pennsylvania State University Department of Poultry Science Seminar, 1984.

Wideman, R. F. Induction of urolithiasis in growing pullets. Agway College Poultry Conference, Syracuse, NY (Invited Presentation), 1984.

Wideman, R. F. The physiology of edema and ascites in poultry. VII Ciclo International de Conferencias Sobre Avicultura, Mexico City (Invited Presentation), 1984.

Wideman, R. F. New findings on kidney damage in pullets and layers. In-service training seminars for Wolgemuth Feed Co. Sales and Service Personnel, The Pennsylvania State University (Invited Presentation), 1985.

Wideman, R. F. Avian phosphate secretion. The Pennsylvania State University Department of Poultry Science Seminar, 1985.

Wideman, R. F. Advances in poultry physiology. Poultry Science Sales and Service Conference, The Pennsylvania State University (Invited Presentation), 1985.

Wideman, R. F. Phosphate excretion by the avian kidney. The Pennsylvania State University Biology Department (Invited Presentation), 1985.

Wideman, R. F. Dose-response of Single Comb White Leghorn pullets to unilateral renal portal citrinin infusion. The Pennsylvania State University Joint Program in Physiology, 1985.

Wideman, R. F. Visceral gout and urolithiasis in poultry. Croton Egg Farms, Inc., Croton, Ohio (Invited Presentation), 1985.

Wideman, R. F. Kidney damage and urolithiasis in poultry. Perdue, Inc., Salisbury, Maryland (Invited Presentation), 1985.

Wideman, R. F. Utilizing the avian renal portal system to evaluate tubular transport. U.S.D.A. Research Labs, Fargo, North Dakota (Invited Presentation), 1985.

Wideman, R. F. The physiology of ascites in broilers. Omeri Poultry Company, Sana, North Yemen, 1985.

Wideman, R. F. The physiology of ascites in poultry. The Pennsylvania State University Department of Poultry Science Seminar, 1985.

Wideman, R. F. Progress report on student recruitment. Poultry Science Industry Council Autumn Meeting, Lancaster, PA, 1985.

Wideman, R. F. Progress in diuresis studies. Perdue, Inc., Salisbury, MD, 1986.

Wideman, R.F., Cowen, B.S., Rothenbacher, H. Pathophysiology of ascites in poultry. Ralston Purina International Ascites Symposium. St. Louis, Missouri (Invited Presentation), 1986.

Wideman, R. F. Renal regulation of avian calcium and phosphorus metabolism. 51<sup>st</sup> Annual Poultry Nutrition Conference, FASEB Meetings, St. Louis (Invited Presentation), 1986.

Wideman, R. F. Diuresis in broiler breeders. Delmarva Hatchery & Breeder Flock Management Short Course. Salisbury, MD (Invited Presentation), 1986.

Wideman, R. F. Dietary control of kidney stones in poultry. Pennsylvania Veterinary-Nutrition Forum, Lancaster, PA (Invited Presentation), 1986.

Wideman, R. F. Diuresis in broiler breeders. The Pennsylvania State University Department of Poultry Science Seminar, 1986.

Wideman, R. F. Alternative animal models to the laboratory rat for biological research: Renal physiology. The Pennsylvania State University Intercollege Graduate Program in Nutrition, Colloquium (Invited Presentation), 1987.

Wideman, R. F. Nutrition and kidney dysfunction in poultry. Maryland Nutrition Conference. Baltimore, MD (Invited Presentations), 1987.

Wideman, R. F. Diuresis in broiler breeder hens. The Pennsylvania State University Poultry Sales and Service Conference. University Park, PA (Invited Presentation), 1987.

Wideman, R. F. Water balance in poultry. Mexican Association of Avian Veterinarians. Monterrey, Mexico (Invited Presentation), 1987.

Wideman, R. F. Diuresis in poultry. VIII Ciclo de Conferencias Internacionales Sobre Avicultura. Mexico City, Mexico (Invited Presentation), 1987.

Wideman, R. F. Wet droppings in poultry. Minnesota Poultry Service Workshop. St. Paul, MN (Invited Presentation), 1987.

Wideman, R. F. Nutrition and kidney damage in poultry. Minnesota Nutrition Conference, Bloomington, MN (Invited Presentation), 1987.

Wideman, R. F. Recent advances in research on the ascites syndrome in broilers. I.C.A. Research Group, Bogota, Colombia (Invited Presentation), 1987.

Wideman, R. F. Recent advances in the study of the ascitic syndrome in broilers. Association of Poultry Nutrition and Veterinary Services, Medellin, Colombia (Invited Presentation), 1987.

Wideman, R. F. Recent advances in the study of the ascitic syndrome in broilers. National Poultry Research Forum, Bogota, Colombia (Invited Presentation), 1987.

Wideman, R. F. Nutrition and kidney dysfunction in poultry. Alabama Egg Industry Conference, Auburn University, Alabama (Invited Presentation), 1987.

Wideman, R.F. Renal regulation of avian calcium and phosphorus metabolism and the urolithiasis syndrome. Poultry Science Department Seminar, University of Georgia, Athens GA (Invited Presentation), 1988.

Wideman, R.F. Assays for calcium-regulating hormones. Poultry Science Department Seminar, University of Georgia, Athens, GA (Invited Presentation), 1988.

Wideman, R.F. Alimet: A successful treatment for urolithiasis. Poultry Sales and Service Conference, University Park, PA (Invited Presentation), 1989.

Wideman, R.F. Pathophysiology of ascites in poultry (5 presentations). Poultry Industry Veterinary and Technical Staff Meetings, Curitiba Brazil; Concordia Brazil; Chapeco Brazil; Florianopolis Brazil; Porto Alegre Brazil (Invited Presentations sponsored by Smith-Kline Animal Health Division), May, 1989.

Wideman, R.F. Pathophysiology of urolithiasis in poultry. Presentation to the faculty and students of the Veterinary Medical School, National University of Costa Rica, San Jose, Costa Rica, June, 1989.

Wideman, R.F. Pathophysiology of urolithiasis in poultry. Presentation to the Costa Rican Association of Poultry Veterinarians and Technicians, San Jose, Costa Rica, June, 1989.

Wideman, R.F. Pathophysiology of ascites in poultry (7 presentations): Carambei Brazil (Batavo); Toledo Brazil (Frigobras-Sadia); Chapeco Brazil (Chapeco group); Chapeco Brazil (COOP Central Oeste Catarinense); Chapeco Brazil (Chapeco-Sadia); Videira Brazil (Perdigao); Caixis de Sul Brazil (Frangosul). Invited Presentations sponsored by Smith-Kline Animal Health Division, August, 1989.

Wideman, R.F. Pathogenia da ascite em frango de corte - O que sabemos? The Eleventh Brazilian Congress on Aviculture. Brasilia, Brasil, (Invited Presentation) August, 1989.

Wideman, R.F. Ascites in poultry. The 5<sup>th</sup> Minnesota Poultry Service Workshop, University of Minnesota, St. Paul, MN (Invited Presentation), September, 1989.

Wideman, R.F. Ascites complications from rapid growth. Pennsylvania Poultry Federation Conference, Grantville, PA (Invited Presentation), September, 1989.

Wideman, R.F. Renal malfunctions: causes and solutions. Tri-State Poultry Federation, Inc. Multi-State Health and Management Conference, Indianapolis, IA (Invited Presentation), October, 1989.

Wideman, R.F. A simplified avian kidney model for studying renal hemodynamics. Physiology Seminar, University of Arkansas, Fayetteville, AR, May, 1990.

Wideman, R.F. A simplified avian kidney model for studying renal hemodynamics. Department of Physiology and Biophysics, College of Medicine, University of Tennessee, Memphis, TN (Invited Presentation), May, 1990.

Wideman, R.F. Integrated calcium homeostasis in laying hens: an overview. Avian Skeletal Disease Symposium, American Association of Avian Pathologists/ AVMA Meeting, San Antonio, TX (Invited Presentation), July, 1990.

Wideman, R.F. Control of renal hemodynamics: the avian renal portal circulation as a model. Symposium on Nonmammalian Models for the study of Cardiovascular Homeostasis. American Physiological Society Fall Meetings, Orlando, FL (Invited Presentation), October, 1990.

Wideman, R.F. The relationship of kidney structure and function to diuresis and urolithiasis. USDA South Central Poultry Research Lab and Mississippi State University Department of Poultry Science, Starkville, MS (Invited Presentation), May, 1991.

Wideman, R.F. Economic aspects of renal function in poultry. Latin American Symposium on Optimizing Poultry Production, Concorde Hotel, Aruba (Invited Presentation), May, 1991.

Wideman, R.F. Economic aspects of renal function in poultry. Symposium of Technological Advances in Poultry Production, Huatulco, Mexico (Invited Presentation), June, 1991.

Wideman, R.F. Urolithiasis: nutrition-disease interactions. Department of Animal Science, College of Agriculture, University of Delaware, Newark, DE (Invited Presentation), December, 1991.

Wideman, R.F. The control of calcium and phosphorus metabolism by the kidneys. Australian Poultry Science Symposium, University of Sydney, Sydney, NSW Australia (Invited to participate as the main guest speaker), February, 1992.

Wideman, R.F. Pathophysiology of ascites. Australian Veterinary Poultry Association Annual Meeting, University of Sydney, Sydney, NSW Australia (Invited Presentation), February, 1992.

Wideman, R.F. Pathophysiology of ascites and The control of calcium and phosphorus metabolism by the kidneys. Parafield Poultry Research Centre, South Australian Department of Agriculture, Adelaide, South Australia, Australia (Invited Presentations sponsored by the Australian branch of the World's Poultry Science Association), February, 1992.

Wideman, R.F. Production and disease problems in commercial egg layers. Victorian Sub-branch of the World's Poultry Science Association, Melbourne, NSW Australia, February, 1992.

Wideman, R.F. Renal regulation of calcium and phosphorus metabolism in domestic fowl. Animal Research Institute, Attwood (Melbourne), NSW Australia (Invited Presentation sponsored by Australian branch of the World's Poultry Science Association), February, 1992.

Wideman, R.F. Trends in broiler production in the United States. New castle Sub-branch of the World's Poultry Science Association, Newcastle, NSW Australia, February, 1992.

Wideman, R.F. Ascites in broilers. New England Sub-branch of the World's Poultry Science Association, Tamworth, NSW Australia, February, 1992.

Wideman, R.F. Pathophysiology of ascites and The control of calcium and phosphorus metabolism by the kidneys. Animal Research Institute, Yeerongpilly (Brisbane), Queensland Australia (Invited Presentations sponsored by the Australian Branch of the World's Poultry Science Association), February, 1992.

Wideman, R.F. Ascites in broilers. Presentations to Management and Service Group, and the Board of Directors of Darwalla Industries, Brisbane, Queensland Australia, February, 1992.

Wideman, R.F. Ascites in broilers. USDA-ARS South Central Poultry Research Lab and Mississippi State University Department of Poultry Science, Starkville, MS (Invited Presentation), June, 1992.

Wideman, R.F. Kidney damage problems affecting poultry production. University of California Poultry Symposium; Modesto, CA: 20 October; Riverside, CA: 22 October (Invited Presentations), 1992.

Wideman, R.F. The influence of nutrition, disease and genetics on flock mortality. University of California Poultry Symposium; Modesto, CA: 20 October; Riverside, CA 22 October (Invited Presentations), 1992.

Wideman, R.F. Twelve years of urolithiasis research. The Pennsylvania State University Department of Poultry Science Seminar, November, 1992.

Wideman, R.F. The role of physiology in improving poultry production. The University of Arkansas Department of Poultry Science Seminar, Fayetteville, AR, 11 March (Invited Presentation), 1993.

Wideman, R.F. Understanding ascites and how to control it. Pennsylvania Poultry Sales and Service Conference, University Park, PA, 6 April, 1993.

Wideman, R. F. Current understanding of the ascites syndrome and future research directions. Novus International Inc. Nutritional and Technical Symposium; Springdale, AR: 16 November; Atlanta GA, 18 November, 1993.

Wideman, R. F. Current research and strategies for controlling ascites. Independence County Poultry Clinic, Arkansas Farm Bureau Federation, Batesville, AR, 23 November, 1993.

Wideman, R.F. Recent advances in ascites research. Arkansas Poultry Symposium, Arkansas Poultry Federation, Springdale, AR, 12 April (Invited Presentation), 1994.

Wideman, R.F. Ascites. Virginia Poultry Health and Management Seminar, Roanoke, VA, 13 April (Invited Presentation), 1994.

Wideman, R.F. Ascites. Mississippi Poultry Association Inc. Poultry Management School, Jackson, MS, 11 May (Invited Presentation), 1994.

Wideman, R.F. Sudden Death Syndrome. Mississippi Poultry Association Inc. Poultry Management School, Jackson, MS, 11 May (Invited Presentation), 1994.

Wideman, R.F. Ascites. Cobb-Vantress Inc., Bear Hollow, MO, 13 June (Invited presentation), 1994.

Wideman, R.F. Ascites in broilers. Arkansas Farm Bureau Poultry Division Meeting, Little Rock, AR, 16 June (Invited presentation), 1994.

Wideman, R.F. Preventing ascites with pulmonary vasodilators. Arkansas Poultry Federation Nutrition Conference, Fayetteville, AR, 14 September (Invited Presentation) 1994.

Wideman, R.F. Proventriculitis. Arkansas Farm Bureau Poultry Research Review, Fayetteville, AR, 5 April (Invited Presentation) 1995.

Wideman, R.F. and D. Rhoads. Developing a RAPD blood test for screening genotypic susceptibility to pulmonary hypertension syndrome (ascites) in poultry. National Breeders Roundtable, St. Louis, MO, 5 May (Invited Presentation) 1995.

Wideman, R. F. Practical research for poultry growers. Arkansas Farm Bureau Poultry Division Meeting, Little Rock, AR, 15 June (Invited Presentation) 1995.

Wideman, R. F. Proventriculitis field studies. CES-BEV/Savoy Broiler Farm Field Day, Savoy, AR, 27 June 1995.

Wideman, R.F. Nutritional and physiological contributions to enlargement and dilation of the proventriculus in broilers. Arkansas Poultry Federation Nutrition Conference, Fayetteville, AR, 13 September (Invited Presentation) 1995.

Wideman, R. F., and D. Rhoads. Developing a DNA based test for screening genotypic susceptibility to pulmonary hypertension syndrome (ascites) in poultry. Sigma Xi Meeting, University of Arkansas Fayetteville Chapter, 6 October (Invited Presentation), 1995.

Wideman, R. F. Overview of current and future ascites research. Hubbard Farms Technical Staff, Walpole, NH, 16 November (Invited Presentation) 1995.

Wideman, R. F. Understanding ascites: the biological basis for developing practical management strategies. Hubbard Farms Symposium for the Arkansas Poultry Industry, Hope, AR, 7 December (Invited Presentation) 1995.

Wideman, R. F. Understanding ascites. Louisiana Poultry Seminar, Shreveport, LA, 13 March (Invited Presentation) 1996.

Wideman, R. F. Metabolic and nutritional interactions in broiler proventriculitis. Roche Animal Nutrition and Health Enteric Disease Conference, 74 Ranch, Campbellton, TX 21-24 March (Invited Presentation) 1996.

Wideman, R. F. Understanding ascites: the biological basis for developing practical management strategies. Poultry Service Veterinarians Seminar, Hedera, Israel 25 June (Invited Presentation) 1996.

Wideman, R. F. El impacto de la arginina sobre la ascitis en pollo de engorda. Fermex/Roche Conference on Technological Advances in Animal Nutrition, Guadalajara, Mexico, 26 September (Invited Presentation) 1996.

Wideman, R. F. El impacto de la arginina sobre la ascitis en pollo de engorda. The 8th Fermex Conference on Synthetic Amino Acids, Mexico City, Mexico, 27 September (Invited Presentation) 1996.

Wideman, R. F. Pulmonary vasodilators and ascites in broilers. The 13<sup>th</sup> Biokyowa Amino Acids Council Meeting, St. Louis, MO, 1 October (Invited Presentation) 1996.

Wideman, R. F. Impact of arginine on ascites in broilers. The 9<sup>th</sup> Biokyowa Amino Acids Meeting in Toronto, Toronto, Ontario, Canada, 10 June (Invited Presentation) 1997.

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## Short communication

# Differential gene expression of proinflammatory chemokines and cytokines in lungs of ascites-resistant and -susceptible broiler chickens following intravenous cellulose microparticle injection

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## ABSTRACT

Intravenous injection of microparticles (MPs) is a tool to reveal susceptibility to pulmonary hypertension (PH) syndrome (PHS, ascites) in broilers. After injection MPs get lodged in pulmonary arterioles and cause localized inflammation. To examine the expression of chemokines/cytokines during the MP-induced pulmonary inflammatory response, lungs were collected from 4-week-old broilers (6/line/time point) from the PHS-resistant (RES) and -susceptible (SUS) broilers before (0 h) and after (2, 6, 12, 24, and 48 h) MP injection and analyzed using quantitative RT-PCR. In both lines, expression of interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-8, and K60 increased from 0 to 6 h, reached peak levels at 6 and 12 h, and decreased thereafter, whereas IL-4 and interferon gamma (IFN- $\gamma$ ) expression remained elevated past 12 h. Lungs from the RES line broilers had higher expression ( $P < 0.05$ ) of IL-1 $\beta$  and IL-6 at 2, 6, and 12 h; higher IL-8 at 6 and 12 h; higher K60 at 6, 12, and 24 h; higher IL-4 at 12, 24, and 48 h and higher IFN- $\gamma$  expression at 6 and 48 h post-MP injection than SUS line broilers. Higher expression of chemokines/cytokines in RES compared to SUS line lungs may explain the ability of RES line broilers to effectively counteract the MP-induced PH and resolve the vascular occlusion.

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## 1. Introduction

Pulmonary hypertension syndrome (PHS, ascites) is a disease of young broilers, characterized initially by pulmonary hypertension (PH), which may lead to ascites and death in susceptible individuals. Intravenous cellulose microparticle (MP) injection is a patented method (US Patent No. 6,720,473) used for screening the susceptibility of broilers to PHS (Wideman and Erf, 2002; Wideman et al., 2002). After being injected i.v. MPs occlude pre-capillary pulmonary arterioles resulting in PH due to increased pulmonary vascular resistance (PVR) (Wideman and Erf, 2002). The MPs entrapped in the lungs initiate an inflammatory response with infiltration and aggregation

of mononuclear cells, consisting of monocytes/macrophages and lymphocytes, in the perivascular region surrounding the arterioles (Wideman et al., 2002; Wang et al., 2003). In our previous study, we examined the infiltration of monocytes/macrophages in the lungs of MP-injected broilers from the ascites-resistant (RES) and -susceptible (SUS) lines of chicken (Hamal et al., 2008; Pavlidis et al., 2007). We found that following i.v. administration of MP, monocyte/macrophage infiltration in the lung tissue of broilers increased within 2 h and continued to do so over the 48 h post-MP injection. This infiltration of monocytes/macrophages was higher in lungs from the RES line broilers than the SUS line broilers (Hamal et al., 2008).

The nature of the observed immune activity associated with MPs lodged in the pulmonary vasculature described above suggests the production of soluble immune-mediators (i.e., chemokines and cytokines). Some of the

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important chemokines and cytokines involved in the initiation and modulation of inflammatory immune activities in chickens include interleukin-8 (IL-8), K60, IL-1 $\beta$ , IL-4, IL-6, and interferon gamma (IFN- $\gamma$ ). Avian and mammalian cytokines are functionally similar even though they are structurally different (Staehele et al., 2001; Giansanti et al., 2006). A proinflammatory role of chemokines IL-8 and K-60 (Withanage et al., 2004), and cytokines IL-1 $\beta$  and IL-6 (Lynagh et al., 2000; Laurent et al., 2001) has been demonstrated in chickens. Similarly, IFN- $\gamma$  has potent macrophage activating capability in mammals and chickens and is the major cytokine driving cell-mediated immune responses to intracellular microbes (Lowenthal et al., 1995; Wigley and Kaiser, 2003), whereas IL-4 is responsible for directing the adaptive immune response towards a humoral response important in the elimination of extracellular antigens and parasites (Howard et al., 1982; Mosmann et al., 1986; Collier et al., 2008).

Using the MP injection model, we hypothesized that chemokines and cytokines would be produced in the lungs of broilers in response to the entrapped MPs and that the time course as well as the balance between the amount and type of chemokines or cytokines produced may play an important role in the pathogenesis of MP-induced PH. Hence, this study was conducted to quantify the expression of chemokines (IL-8 and K60) and cytokines (IL-1 $\beta$ , IL-6, IL-4, and IFN- $\gamma$ ) in the lungs of broilers from RES and SUS lines at 0, 2, 6, 12, 24, and 48 h post-injection of MP.

## 2. Materials and methods

### 2.1. Broiler management

All procedures involving broiler chickens were approved by the University of Arkansas Institutional Animal Care and Use Committee. The broilers from the RES and SUS genetic lines used for this study were developed by divergent selection and rearing them in a hypobaric chamber. At the time of this study the lines were at the ninth generation of their selection and exhibited ascites mortalities of 7.5% (RES line) and 75% (SUS line) when reared under hypobaric conditions (Pavlidis et al., 2007).

Fifty newly hatched male chicks per line from RES and SUS lines were reared on fresh wood shavings in environmental chambers (8 m<sup>2</sup> floor space). Chicks were brooded at 33.2 °C on d 1–3, at 31.1 °C on d 4–6, at 29.4 °C on d 7–10, at 25.5 °C on d 11–14, and at 23.9 °C, d 15 onwards. Feed and water were provided ad libitum. Light schedules were 24 h/d for d 1–4, and 16 h light and 8 h dark from d 5 onwards.

### 2.2. Microparticle injection and tissue collection

Fifty male broilers (4-week-old) per line were injected with MP as described previously (Wideman and Erf, 2002; Wang et al., 2003). Briefly, micro-granular CM-32 ion exchange cellulose (Fisher Scientific, St. Louis, MO) was suspended at the rate of 0.02 g/mL in a heparinized saline solution. This suspension was injected into the broilers via the left wing vein at the dose of 0.35 mL/

broiler using a 1-mL tuberculin syringe (Becton Dickinson, Franklin Lake, NJ).

The right lung was collected from six broilers per line at 0 h (MP un-injected control lung) and 2, 6, 12, 24, and 48 h post-MP injection. The lung tissue was sliced into 5–6 small pieces and then immersed into 3 mL of RNeasy lysis buffer (Qiagen Inc., Valencia, CA). An adjacent segment from each lung was immersed in 10% buffered formalin, embedded in paraffin, sectioned at 5  $\mu$ m, stained with hematoxylin and eosin, and assessed microscopically to confirm that each of the lungs collected at 2, 6, 12, 24, and 48 h post-injection had entrapped substantial numbers of MPs.

### 2.3. Real-time RT-PCR (reverse transcription-polymerase chain reaction)

#### 2.3.1. RNA isolation

RNA was isolated from the RNeasy stored lung tissues using the Aurum<sup>TM</sup> total RNA fatty and fibrous tissue kit (Bio-Rad, Hercules, CA) and following the spin format protocol with slight modification. For RNA isolation 91–99  $\mu$ g of lung tissue per sample was used. To remove any contaminating DNA, an additional DNA digestion step was performed. The RNA samples were aliquoted into four subsamples and stored at –80 °C until analysis.

#### 2.3.2. Assessment of quality and quantity of RNA

The quality and quantity of the RNA were assessed using an Experion automated electrophoresis system and the Experion<sup>TM</sup> RNA StdSens analysis kit (Bio-Rad) following the manufacturers' protocol with some modifications. Automated electrophoresis provided a virtual gel and an electropherogram showing intact 18S and 28S band peak indicating good quality RNA.

#### 2.3.3. Reverse transcription

RNA (2.5  $\mu$ g/sample) was reverse transcribed to cDNA using Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA). Briefly, 40  $\mu$ L of each reaction mixture contained 4  $\mu$ L of 10 $\times$  RT buffer, 1.6  $\mu$ L of 25 $\times$  dNTP mix (100 mM), 4  $\mu$ L of 10 $\times$  RT random primers, 2  $\mu$ L of MultiScribe reverse transcriptase (50 U/mL), and 2.5  $\mu$ g total RNA with nuclease free H<sub>2</sub>O added to bring the volume to 40  $\mu$ L. The incubation steps used were: one cycle of 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s. After cDNA synthesis, the cDNA samples were aliquoted into four subsamples of 10  $\mu$ L each and stored at –80 °C until analysis.

#### 2.3.4. Quantification of relative gene expression of chemokines and cytokines

Real-time PCR was performed using TaqMan Universal PCR master mix in an ABI PRISM 7300 sequence detection system (Applied Biosystems). The PCR was performed in a reaction volume of 25  $\mu$ L containing the reagents at the following final concentrations: 1 $\times$  TaqMan Universal PCR master mix (2 $\times$ ), forward primer 200 nM, reverse primer 200 nM, Probe 100 nM, and 1  $\mu$ L of cDNA sample. The cycling profiles used were: 1 cycle at 50 °C for 2 min, 95 °C for 10 min, and 40 cycles (95 °C for 15 s, and 60 °C for 60 s).

**Table 1**

Primers and probe sequences used for real-time RT-PCR.

Target	Primer or probe <sup>a</sup>	Sequences	Exon boundary	Genbank accession #
28S <sup>b</sup>	Forward Reverse Probe	5'-GGCGAAGCCAGAGGAACT-3' 5'-GACGACCGATTTCACGTC-3' 5'-AGGACCGCTACGGACCTCCACCA-3'		X59733
IL-1 $\beta$ <sup>b</sup>	Forward Reverse Probe	5'-GCTCTACATGTCGTGTGTATGAG-3' 5'-TGTCGATGTCCCGCATGA-3' 5'-CCACACTGCAGCTGGAGGAAGCC-3'	5/6	AJ245728
IL-4 <sup>c</sup>	Forward Reverse Probe	5'-AACATGCGTCAGTCTCTGAAT-3' 5'-TCTGCTAGGAACCTCTCCATTGAA-3' 5'-AGCAGCACCTCCCTCAAGGCACC-3'	3/4	AJ621735
IL-6 <sup>b</sup>	Forward Reverse Probe	5'-GCTCGCCGGCTTCGA-3' 5'-GGTAGGTCTGAAAGGCCGAACAG-3' 5'-AGGAGAAATGCCTGACGAAGCTCTCCA-3'	3/4	AJ250838
IL-8 <sup>b</sup>	Forward Reverse Probe	5'-GCCCTCCTCTGGTTTCAG-3' 5'-TGGCACCGCAGCTCATT-3' 5'-TCTTTACCAGCGTCTACCTTGCGACA-3'	1/2	AJ009800
IFN- $\gamma$ <sup>b</sup>	Forward Reverse Probe	5'-GTGAAGAAGGTGAAAGATATCATGGA-3' 5'-GCTTTGCGCTGGATTCTCA-3' 5'-TGGCCAAGCTCCCGATGAACGA-3'	3/4	Y07922
K60 <sup>d</sup>	Forward Reverse Probe	5'-TGATGGGCAAGGCTGTAGCT-3' 5'-GTGCTGAGCCATACCTTTTG-3' 5'-TCATGGCTCTTCTCTGATCTCAATGGCT-3'	1/2	AF27760

<sup>a</sup> Probes had FAM (6-carboxyfluorescein) at 5' end and TAMRA (6-carboxytetramethylrhodamine) at 3' end.<sup>b</sup> Previously published primers and probes (Kaiser et al., 2003).<sup>c</sup> Previously published primers and probes (Rothwell et al., 2004).<sup>d</sup> Primers and probes designed by author.

Previously published primers and probes for 28S and chicken IL-1 $\beta$ , IL-6, IL-8, IFN- $\gamma$ , and IL-4 were used for PCR (Kaiser et al., 2003; Rothwell et al., 2004). The primers and probes for K60 were designed by the author using the Primer Express software (version 2.0, Applied Biosystems). The sequences for the primers and probes used are listed in Table 1. In each plate, a no template control (no cDNA, master mix only), a calibrator sample, cDNA samples, and endogenous control (28S) were included. Endogenous control (28S) samples were analyzed in duplicate and the target genes were analyzed in triplicate. The calibrator sample was cDNA from the lung samples from the relaxed line that had not been injected with MP. The relative gene expression was quantified by the delta delta Ct method. The fold change in gene expression was calculated by comparing the gene expression of the sample with the expression of the calibrator sample, which was the MP un-injected sample.

#### 2.4. Statistical analysis

Using JMP statistical software (version 7.0.1, SAS Institute, Cary, NC, USA), one-way ANOVA was carried out to determine differences in the fold change of target genes in lungs of broilers from the RES and SUS lines at a given time point and between samples collected at the various time points within the RES or SUS line. Differences among the group means were determined by Fisher's LSD multiple mean comparisons test. Data were expressed as mean  $\pm$  SEM, and the differences considered significant at  $P \leq 0.05$ .

### 3. Results and discussion

#### 3.1. Chemokines (IL-8 and K60)

IL-8 expression increased in the lungs of broilers from the RES and SUS lines in response to MP injection as early as 2 h post-injection (6-fold increase in the RES line vs. 5.5-fold increase in the SUS line, Fig. 1a). In the SUS line, IL-8 expression did not increase further after 2 h but instead plateaued at the 2 h-level until 12 h and then decreased thereafter. In contrast IL-8 expression in the RES line continued to increase beyond 2 h, peaked by 6 and 12 h (9-fold increase) and then started to decrease thereafter. Lungs from the RES line broilers had higher expression of IL-8 at 6 h ( $P = 0.005$ ) and 12 h ( $P = 0.005$ ) when compared with the lungs from the SUS line broilers. IL-8 expression in the two lines did not differ at other time points (Fig. 1a).

K60 expression increased by 2 h post-MP injection in the lungs of broilers from the RES and the SUS lines (7.5-fold increase in the RES line vs. 9-fold increase in the SUS line, Fig. 1b). In the SUS line, K60 expression did not increase after 2 h but instead remained at the same level until 12 h and started to decrease thereafter. In the RES line K60 expression continued to increase beyond 2 h and peaked by 6 h (13-fold increase), remained at same level at 12 h and started to decrease thereafter. Lungs from RES line broilers had higher expression of K60 at 6 h ( $P < 0.0001$ ), 12 h ( $P = 0.0074$ ), and 24 h ( $P = 0.023$ ) when compared with the lungs from SUS line broilers. K60 expression in the two lines did not differ at other time points (Fig. 1b).

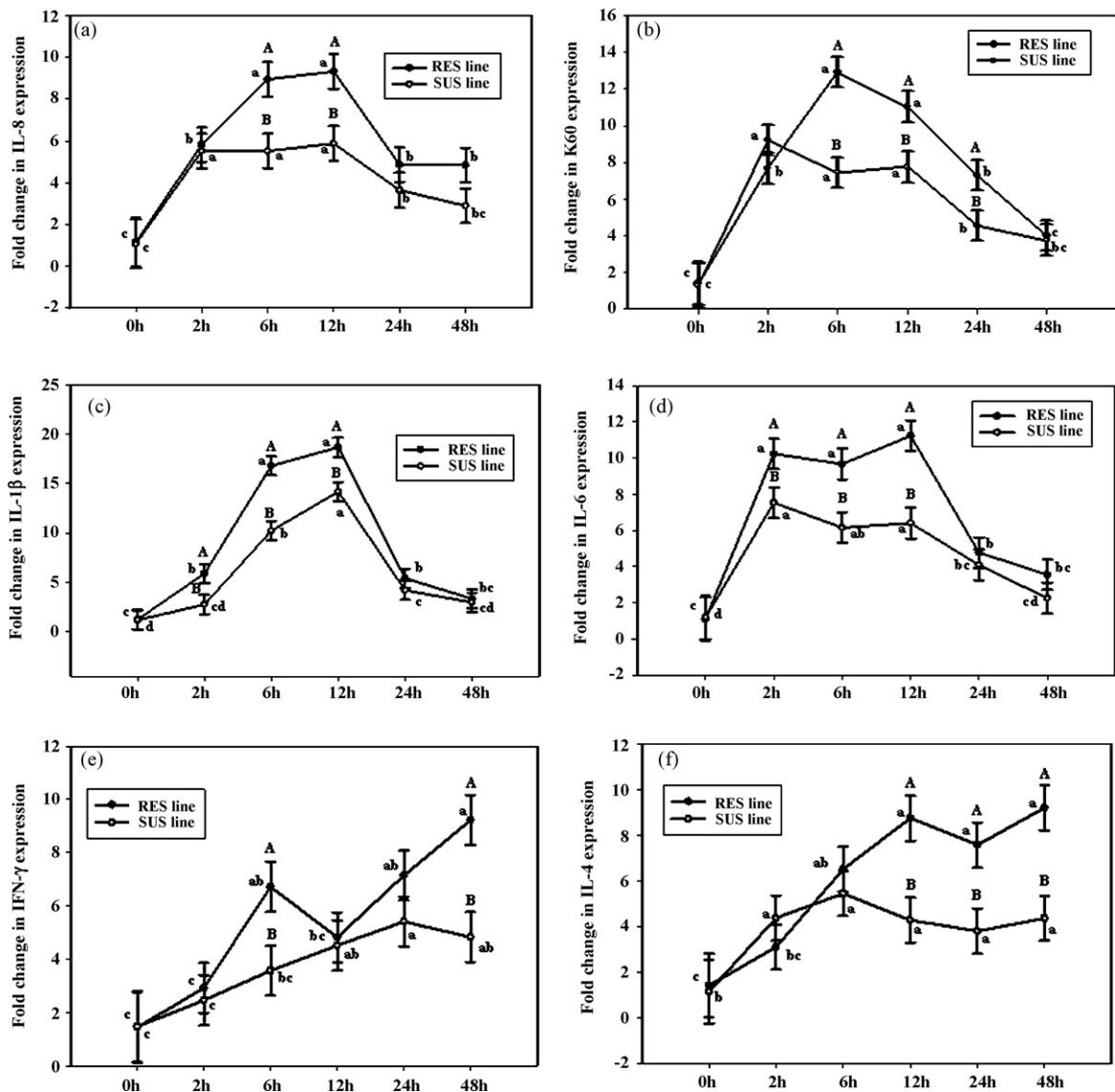


Fig. 1. Fold change in gene expression of interleukin-8 (IL-8, a), chemokine K60 (b), IL-1 $\beta$  (c), IL-6 (d), interferon gamma (IFN- $\gamma$ , e), and IL-4 (f) in lungs of broilers from the ascites-resistant (RES) and -susceptible (SUS) lines at 0 (microparticle not-injected), 2, 6, 12, 24, and 48 h post i.v. microparticle injection. Relative gene expression was quantified by real-time reverse transcription PCR using an Applied Biosystems 7300 sequence detection system. The fold change in gene expression was computed by the delta delta ( $\Delta\Delta$ ) Ct method, where MP un-injected cDNA sample was used as the calibrator and 28S cDNA served as an endogenous control. Data were expressed as means  $\pm$  SEM ( $n = 6$  per line and time point). Letters A and B indicate line differences between each time point; letters a, b, c, and d indicate differences among the time points within each line. For each analysis, means without a common letter are different ( $P \leq 0.05$ ).

Rapid increase in the expression of the chemokines IL-8 and K60 in the lungs of broilers from both lines following MP injection suggests that acute inflammation takes place in the lungs in response to MPs entrapped in the pulmonary vasculature (Wideman et al., 2002; Wang et al., 2003). The main biological function of chemokines is facilitation of the migration of immune cells to the site of infection or injury (Laing and Secombes, 2004). Higher expression of these chemokines in the lungs from the RES line than in the SUS line would suggest that these chemokines enable the RES line broilers to recruit the leukocytes more efficiently.

### 3.2. Proinflammatory cytokines (IL-1 $\beta$ and IL-6)

IL-1 $\beta$  expression increased in the lungs of broilers from the RES and SUS lines in response to MP injection (Fig. 1c). IL-1 $\beta$  expression increased within 2 h post-injection of MP in the RES line (6-fold increase) and within 6 h in the SUS line (10-fold increase, Fig. 1c). IL-1 $\beta$  expression remained elevated in both lines until 12 h post-injection (19-fold increase in the RES line vs. 14-fold increase in the SUS line, Fig. 1c). By 24 h IL-1 $\beta$  expression decreased and remained at that level by 48 h in both lines. Lungs from the RES line broilers had higher expression of IL-1 $\beta$  at 2 h ( $P = 0.025$ ),

6 h ( $P < 0.001$ ), and 12 h ( $P = 0.0017$ ) compared to the lungs from the SUS line broilers. IL-1 $\beta$  expression in the two lines did not differ at the remaining time points (Fig. 1c).

IL-6 expression increased in the lungs of broilers from the RES and SUS lines in response to MP injection (Fig. 1d). In both lines the expression of IL-6 increased rapidly by 2 h post-injection (10-fold increase in RES line vs. 7.5-fold increase in SUS line), remained at elevated levels until 12 h and started to decrease by 24 h onwards (Fig. 1d). Lungs from RES line broilers had higher expression of IL-6 at 2 h ( $P = 0.028$ ), 6 h ( $P = 0.0049$ ), and 12 h ( $P = 0.0001$ ) compared to the lungs from SUS line broilers. IL-6 expression in the two lines did not differ at other time points (Fig. 1d).

Similar to chemokines, there was rapid increase in the expression of the proinflammatory cytokines (IL-1 $\beta$ , IL-6), in the lungs of broilers in response to entrapped MPs, which indicates that the MPs initiate an inflammatory response in the lung. IL-1 $\beta$  and IL-6 are produced by activated phagocytes, especially macrophages, as well as endothelial cells (Van Snick, 1990; Lederer and Czuprynski, 1995). They are particularly important in the early phase of the innate immune response, recruiting and activating leukocytes and setting the stage for appropriate action in response to microbes, tissue injury, and other components that signal danger. Hence, it is not surprising that expression of these cytokines returned to near baseline levels by 24 h post i.v. MP injection. Once activated, leukocytes that have come to the area of inflammation (e.g., macrophages, lymphocytes) and/or local cells (e.g., mast cells, endothelial cells) focus on taking appropriate action to effectively eliminate the microbes/foreign particles, repair the tissue damage, and resolve the inflammation.

### 3.3. Interferon gamma (IFN- $\gamma$ ) and IL-4

IFN- $\gamma$  expression increased in the lungs of broilers from the RES and SUS lines in response to the MP injection (Fig. 1e). IFN- $\gamma$  expression increased by 6 h post-injection (6.7-fold increase) in the RES line and by 12 h post-injection (4.5-fold increase) in the SUS line, and remained at elevated level at 24 and 48 h post-injection in both lines. Lungs from the RES line broilers had higher expression of IFN- $\gamma$  at 6 h ( $P = 0.021$ ) and 48 h ( $P = 0.0016$ ) when compared with the lungs from SUS line broilers. IFN- $\gamma$  expression in the two lines did not differ at other time points (Fig. 1e).

IL-4 expression increased in the lungs of broilers from the RES and SUS lines in response to MP injection (Fig. 1f). IL-4 expression increased by 6 h post-injection (6.5-fold increase) in the RES line and by 2 h post-injection (4.3-fold increase) in the SUS line, and remained at elevated level thereafter in both lines. Lungs from the RES line broilers had higher expression of IL-4 at 12 h ( $P = 0.002$ ), 24 h ( $P = 0.009$ ), and 48 h ( $P = 0.001$ ) when compared with the lungs from the SUS line broilers. The two lines did not differ in IL-4 expression at the remaining time points (Fig. 1f).

Cytokines like IFN- $\gamma$  and IL-4 can greatly heighten the activities of inflammatory cells and direct the innate and adaptive immune activities to effectively respond to intracellular and endogenous antigens or extracellular

antigens, respectively. In this study expression of these cytokines was evident by 6 h at which time it is unlikely that they would originate from antigen-activated T helper cells as a result of a primary adaptive immune response. In humans, it is well established that natural killer (NK) cells are the chief source of IFN- $\gamma$  during the early immune response (Hussell and Openshaw, 1998; Fehniger et al., 1999; Merlino and Marsh, 2001). Although there is no direct evidence of avian NK cells secreting IFN- $\gamma$  in the early phase of the immune response, considering the influx of CD8<sup>+</sup> lymphocytes in the periarteriolar region (Wang et al., 2003) it is likely that the source of IFN- $\gamma$  is the NK cell (CD8<sup>+</sup>). Similarly, during the 48 h post i.v. MP injection, the source of IL-4 is likely the tissue mast cell/infiltrating basophils which may have been activated by factors of innate immunity such as the slow reacting substances of anaphylaxis produced during the complement cascade. To the best of our knowledge it is not known whether the chicken basophils secrete IL-4 but human basophils are known to secrete IL-4 (Aumüller et al., 2003). Additionally basophil infiltration around the MP occluded pulmonary arterioles in broiler lung has been reported previously (Wang et al., 2003). Therefore it is very likely that basophils may be the source of IL-4 especially during the first 48 h post i.v. MP injection.

IFN- $\gamma$  and IL-4 expression increased in lungs of both the RES and SUS line broilers for the first 24 h post-MP injection, after which time IFN- $\gamma$  expression leveled off in the SUS line but continued to increase in the RES line. Moreover, expression of these cytokines was higher in the RES line, especially at the later time points examined (Fig. 1e and f). This observation also suggests a more effective and sustained ability of the RES line to mount an effective inflammatory response to the pulmonary challenge imposed by the i.v. MP injection. IFN- $\gamma$  mediated macrophage activation may lead to the production of vasodilators and factors involved in tissue destruction and repair, whereas IL-4 activation of mast cells and basophils may lead to the production of amines, proteins and lipid mediators. All these factors may play a role in modulating blood flow and dislodging and killing of large parasites in tissues, which would be helpful in dealing with MPs lodged in arterioles in an effort to restore normal function of the lung.

The differential expression of these chemokines and cytokines in lungs of the MP-injected RES and SUS line broilers may be due to the differences in the number, type, and activity of the infiltrating cell population. Our previous study demonstrated that monocytes/macrophages infiltration in the lungs of MP-injected broilers increased consistently from 0 to 48 h post-injection of MP with lungs from the RES line broilers having higher infiltration of monocytes/macrophages than the SUS line (Hamal et al., 2008). As monocytes/macrophages are a major source of the proinflammatory cytokines and chemokines examined here, the higher and more sustained expression of these cytokines and chemokines in the RES lungs appears to be due to the higher numbers of activated monocytes/macrophages responding to the occlusion of arterioles by MP. Preliminary examination of lung-infiltrating lymphocyte populations using immu-

nohistochemical staining and image analysis revealed that i.v. MP injection of RES and SUS broilers resulted in increased infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> cells by 24 h post-MP injection ( $P < 0.05$ ), but the extent of infiltration was not different in lungs of RES and SUS line broilers (data not shown). With similar amounts of CD4- and CD8-defined cells responding to the vascular occlusion, their potential contributions to IFN- $\gamma$  or IL-4 expression may reflect heightened activity of these cells in the RES compared to the SUS line broilers. The cellular source of IFN- $\gamma$  and IL-4 this early in the inflammatory response of broilers needs to be further examined.

In conclusion, examination of chronological, quantitative, and qualitative aspects of the pulmonary chemokine and cytokine expression during the first 48 h post i.v. MP injection suggests that the innate immune activity initiated in the RES broiler plays an important role in its ability to survive and effectively cope with the MP challenge. Further studies are needed to determine the cells and mechanisms involved in driving this inflammatory response that allows survival during the critical 2–48 h post i.v. MP injection and ultimately restoration of normal function and capacity of the lung.

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# Intratracheal Administration of Bacterial Lipopolysaccharide Elicits Pulmonary Hypertension in Broilers with Primed Airways

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**ABSTRACT** Broilers reared under commercial conditions inhale irritant gases and aerosolized particulates contaminated with gram-negative bacteria and bacterial lipopolysaccharide (LPS). Previous studies demonstrated that i.v. injections of LPS can trigger an increase in the pulmonary arterial pressure (PAP); however, the pulmonary hemodynamic response to aerosolized LPS entering via the most common route, the respiratory tract, had not been evaluated in broilers. In experiment 1, broilers reared on new wood shavings litter in clean environmental chambers either were not pretreated (control group) or were pretreated via aerosol inhalation of substances (food color dyes and propylene glycol) known to sensitize the airways. One day later, the broilers were anesthetized, catheterized to record the PAP, and an intratracheal aerosol spray of LPS (1 mL of 2 mg/mL of LPS) was administered. Broilers in the control group as well as broilers

pretreated with aerosolized distilled water or yellow and blue food color dyes did not develop pulmonary hypertension (PH; an increase in PAP) after the intratracheal spray of LPS, whereas broilers that had been pretreated with red food color did develop PH in response to intratracheal LPS. In experiment 2, birds raised under commercial conditions on used wood shavings litter developed PH in response to intratracheal LPS regardless of whether they had been pretreated with aerosolized red food color dye. In experiment 3, broilers reared in clean environmental chambers on new wood shavings litter were used to demonstrate that Red Dye #3 and propylene glycol are capable of priming the responsiveness of the airways to a subsequent intratracheal LPS challenge. Common air contaminants such as LPS can result in PH leading to pulmonary hypertension syndrome (ascites) in broilers with appropriately primed airways.

**Key words:** lipopolysaccharide, broiler, mucosal immune system, air pollutant

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## INTRODUCTION

Bacterial lipopolysaccharide (LPS; endotoxin) is an integral component of the cell wall of gram-negative bacteria (e.g., *Escherichia coli* and *Salmonella*). Animals in commercial production facilities are chronically challenged with airborne gram-negative bacteria and LPS. Approximately 40% of the gram-negative bacteria and LPS in inhaled dust reside on particles of a respirable size ( $\leq 5 \mu\text{m}$  in diameter) capable of penetrating to the gas exchange parenchyma and triggering an inflammatory response that is profoundly deleterious to respiratory function. Symptoms of respiratory inflammation that cumulatively attenuate the growth and productivity of domesticated animals include dyspnea (labored breathing due to elevated airway resistance), hypoxemia (undersaturation of arterial blood with oxygen), and hemodynamic dysfunction including pulmonary arterial hypertension (PAH)

and reduced cardiac output due to pulmonary vasoconstriction (Anderson et al., 1966; Hayter and Besch, 1974; Gross, 1990; Whyte, 1993; Sander, 1994; Brown et al., 1997; Canonico and Brigham, 1997; Parsons et al., 1997; Reynolds, 1997; Tottori et al., 1997; Fedde, 1998; Yamaguchi et al., 2000; Zucker et al., 2000; Alexander and Rietschel, 2001; Bakutis et al., 2004).

Most aerosol particulates and pathogens are trapped by mucus in the conducting airways and are prevented from entering the lung parenchyma by the mucociliary escalator. Susceptibility to airborne particulates increases when mucociliary transport is inhibited by exposure to  $\text{NH}_3$  and infectious bronchitis virus or when aerosolized particles are small enough to be conveyed with the inspired air to the terminal gas exchange surfaces. Respirable particulates reaching the lung parenchyma must penetrate the surfactant layer before being engulfed by phagocytic gas-exchange epithelial cells and translocated to the interstitium where LPS can bind to receptors on the surface of monocytes, heterophils, thrombocytes, and endothelial cells (Stearns et al., 1987; Brown et al., 1997). The ensuing cascade of intracellular signaling events culminates in the following: transcription and translation of

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genes associated with the innate immune response, production and local release/expression of inflammatory cytokines, production of the vasodilator NO, and production or release of vasoconstrictors including thromboxane and serotonin (Brown et al., 1997; Wideman et al., 2004).

Broiler chickens are sensitive to respiratory perturbations, because their lung capacity is only marginally adequate to support optimal performance under the best of conditions. Accordingly, broilers are susceptible to the onset of hypoxemia and PAH contributing to pulmonary hypertension syndrome (ascites) whenever vasoconstrictors released during the innate immune response overwhelm concurrently produced vasodilators. Intravenous LPS injections trigger PAH that, on average, is delayed in onset by 10 to 15 min, attains the major peak response within approximately 20 to 25 min postinjection, and thereafter gradually recedes toward the baseline pressure. These responses are not caused by contemporaneous changes in cardiac output; therefore, the hypertensive phase reflects net pulmonary vasoconstriction, whereas the subsequent recovery phase reflects net pulmonary vasodilation. Variation among broilers in their responses to LPS likely reflects variation in the relative proportions or profiles of vasoconstrictors and vasodilators produced during the inflammatory cascade (Wideman et al., 2001, 2004; Wang et al., 2002a,b, 2003; Wideman and Chapman, 2004; Chapman et al., 2005; Bowen et al., 2005a,b).

Pulmonary hemodynamic responses to airborne (aerosol) LPS may differ substantially from those triggered by i.v. LPS injections. Airway defense mechanisms potentially can minimize the interaction of inhaled LPS with the leukocytes responsible for producing key vasoconstrictors, whereas i.v. LPS administration provides immediate and intimate access to the innate immune system. A previous experiment demonstrated that respiratory function was compromised in broilers reared on floor litter where they inhaled litter dust and fumes, when compared with broilers reared in clean stainless steel cages (Wang et al., 2002b). However, prolonged exposure of broilers to floor litter is an imprecise method for evaluating time-course responses to LPS. Accordingly, the present studies were conducted to pursue the development of a suitably controllable model for exposing the conducting airways and gas exchange parenchyma of broiler lungs to aerosolized LPS.

## MATERIALS AND METHODS

### *Bird Management*

Broiler chicks were wing-banded and housed in environmental chambers (8 m<sup>2</sup> of floor space) within the Poultry Environmental Research Lab (PERL) on the University of Arkansas Poultry Research Farm. The birds were brooded at 33°C from d 1 to 3, 31°C from d 4 to 6, 29°C from d 7 to 10, 26°C from d 11 to 14, and 24°C thereafter. Birds were fed a 23% CP corn-soybean meal-based diet formulated to meet the NRC (1994) standards for all ingredients. Feed and water were provided ad libitum. Feed

was provided as crumbles throughout the experiment. Lights were on for 24 h/d through d 4 and 16 h/d thereafter.

### *Pilot Aerosol Delivery Technique Studies*

Preliminary studies were conducted to evaluate methods for delivering aerosolized LPS into the respiratory system. All aerosol exposures to LPS were conducted inside a fume hood within the PERL. The LPS from *Salmonella* Typhimurium (Sigma Chemical Co., St Louis, MO) was dissolved in 0.9% sodium chloride solution (9 g of NaCl/L). Broiler weights were recorded before and 24 h after LPS was administered. Aerosolized LPS delivery was considered to be effective when the responses of the birds were typical of those known to be triggered by i.v. LPS injections, including lethargy, depression, avoidance of feed and water, and minimal or negative weight gain over a 24-h period (Xie et al., 2000; Wang et al., 2003). For each method of aerosol delivery assessed, a subset of additional experiments was conducted in which LPS was replaced with red food color dye (McCormick Food Colors and Egg Dye, McCormick & Co. Inc., Hunt Valley, MD). Necropsies were carried out on 2 birds from each treatment to qualitatively assess the penetration of the aerosolized food color into the trachea, bronchi, parabronchi, and air sacs.

**Passive Aerosol LPS Inhalation.** A CompMist Piston Compressor Nebulizer (Mabis Healthcare, Waukegan, IL) was used to vaporize the LPS solution into aerosol droplets ranging in diameter from 0.5 to 10  $\mu$ m (Tiano and Dalby, 1996; Rau, 2002). During evaluations using distilled water, this nebulizer aerosolized approximately 0.25 mL of solution per min. Broilers (n = 15, 19 d of age; 357  $\pm$  8 g of BW) were manually supported in an upright posture with their head inserted through a suitably sized opening in a Plexiglas box (30  $\times$  20  $\times$  30 cm; length  $\times$  width  $\times$  height) facing the outlet tube from the nebulizer (head-directed aerosol delivery). The 2-cm diameter outlet tube was positioned 10 cm from the face of the bird to direct vaporized LPS (6 mg of LPS/10 mL) toward the mouth and nostrils of the bird for 1 to 4 min. The Plexiglas box prevented air currents in the fume hood from diverting the aerosol away from the face of the bird. When food color was substituted for the LPS, red aerosol vapor was observed to streamline into the mouth and nostrils of the bird as it inhaled, and some red vapor exited the nostrils during expiration. In a second approach designed to permit long intervals of aerosol exposure for multiple birds, up to 4 broilers at a time were placed inside an 80-L<sup>3</sup> plastic box (50  $\times$  40  $\times$  40 cm), and the outlet tube from the nebulizer was inserted through a 2-cm hole in the uppermost portion of 1 end of the box. Aerosolized LPS was allowed to suffuse the entire box for up to 40 min (whole-body aerosol delivery). The CO<sub>2</sub> accumulation inside the box promoted vigorous panting, which facilitated deep inhalation of the fog-like LPS vapor into the respiratory tract. The nebulizer was loaded with LPS (6 mg of LPS/10 mL) and was refilled as needed to permit 40 min

of whole-body aerosol LPS delivery ( $n = 12$ ; 19 d of age;  $596 \pm 16$  g of BW). For both the head-directed and whole-body LPS aerosol delivery techniques, the test broilers rarely exhibited any reduction in 24-h BW gain when compared with nonexposed flock mates (data not shown). Necropsies revealed food color aerosolized by both techniques throughout the conducting airways and within the parabronchi. It was evident throughout these pilot studies that it would be difficult to determine the quantity of LPS inhaled and retained during passive aerosol inhalation and that aerosol LPS inhalation would be difficult to administer while cardio-pulmonary hemodynamic variables were being recorded in broilers.

**Direct Intratracheal Aerosol LPS Injection.** For direct intratracheal administration of LPS, an IA-1B MicroSprayer (17.5-cm cannula length; Penn-Century Inc., Philadelphia, PA) connected to a 1-mL syringe was used to spray aerosolized LPS directly into the lower portions of the trachea, as described elsewhere for mammals (Wheelodon et al., 1992; Sookhai et al., 2002; Bivas-Benita et al., 2005). Broilers were restrained in dorsal recumbency inside the fume hood, the oral cavity was opened, and the larynx was visualized by carefully retracting the tongue. The cannula of the MicroSprayer was introduced into the trachea with the tip of the cannula located proximal to the syrinx, then 1 mL of LPS (2 mg/mL) was injected as an aerosol spray. Immediately after injecting the LPS, the MicroSprayer was withdrawn from the trachea, and the head of the bird was held upward with the beak held closed to ensure the LPS spray remained within the airways. Food color administered by this technique penetrated into the parabronchi. Birds receiving the intratracheal aerosol LPS spray ( $n = 11$ , 35 d of age;  $1,929 \pm 21$  g of BW) tended to be depressed and lethargic during the ensuing 6 h, and they exhibited minimal or no 24-h BW gain (data not shown). Based on this evidence of a biological response to LPS, the intratracheal aerosol LPS delivery technique was used for subsequent experiments.

### Pilot PAP Studies

Five broilers inhaled aerosolized red food color for 40 min via the whole-body delivery technique but were not used to assess the anatomical distribution of the inhaled dye. These preaerosolized broilers (36 d of age;  $2,744 \pm 115$  g of BW) were used within 24 h of postaerosol exposure along with an equal number of their nonaerosolized control flock mates (37 d of age;  $2,808 \pm 87$  g of BW) in pilot studies to determine if the intratracheal aerosol LPS spray would cause the PAP to increase. All birds were anesthetized using i.m. injections of 1 mL of allobarbitol (5,5-diallyl-barbituric acid; 25 mg/mL; Sigma Chemical Co.) and 1 mL of ketamine HCl (100 mg/mL). They were fastened in dorsal recumbency on a surgical board. Lidocaine HCl (2%) was injected s.c. around the basilica vein, then the proximal end of a Silastic catheter (0.012 in i.d., 0.025 in o.d.) filled with heparinized saline (200 IU of heparin/mL of 0.9% NaCl) was inserted into the vein. The distal end of the catheter was attached to a blood

pressure transducer interfaced through a Transbridge pre-amplifier (World Precision Instruments, Sarasota, FL) to a Biopac MP100 data acquisition system using AcqKnowledge software (Biopac Systems Inc., Santa Barbara, CA). The catheter was slowly advanced into the basilica vein, right atrium, right ventricle, and main trunk of the pulmonary artery where PAP was recorded as described previously (Guthrie et al., 1987; Wideman et al., 1996; Wideman, 1999). Birds were allowed to stabilize for a period of 8 min, and control PAP data were recorded for 4 min. One milliliter of LPS (2 mg/mL) was administered using the intratracheal MicroSprayer as described above, and the PAP was recorded for an additional 44 min. Approximately 20 min after LPS administration, the PAP increased above control levels in 4 out of 5 broilers that had been pretreated with aerosolized red food color and in only 1 out of 5 of the nonaerosolized control broilers.

These unanticipated observations led to a series of experiments designed to replicate the initial phenomenology and to identify the compound(s) in the red food color responsible for apparently sensitizing or priming the respiratory tract of broilers to LPS. The whole-body aerosol delivery technique was used for 40 min to deliver aerosolized distilled water, McCormick food colors, or saturated aqueous solutions of some of the primary ingredients listed for these food colors, including FD&C Red #3 and the carrier solvent propylene glycol (PG). Various dye compounds including FD&C Red #3 and FD&C Red Dye #40, and carrier solvents including PG, have been implicated as agents responsible for priming or amplifying respiratory inflammatory and allergic (bronchial-constrictive) responses in humans and experimental mammals (Fisherman and Cohen, 1973; Michaelsson and Juhlin, 1973; Weber et al., 1979; Pruitt, 1985; Berlin, 1997). Three experiments were conducted in which broilers were anesthetized and prepared for PAP recordings approximately 24 h postexposure to whole-body aerosol delivery of distilled water, food colors, or their ingredients.

**Experiment 1.** Male broilers from a commercial source were wing-banded and reared on clean wood shavings litter in environmental chambers within the PERL. When they were 43 to 55 d of age, the broilers were randomly assigned to 1 of 5 groups: nonaerosolized broilers (NA;  $n = 11$ ;  $3,051 \pm 75$  g of BW) were not previously exposed to aerosol inhalation and were injected i.v. with 1 mL of 2 mg/mL of LPS while the PAP was recorded; water-aerosolized broilers (WA;  $n = 10$ ;  $3,014 \pm 103$  g of BW) inhaled aerosolized distilled water for 40 min then 24 h later received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray; red food color-aerosolized broilers (RA;  $n = 13$ ;  $2,929 \pm 81$  g of BW) inhaled aerosolized McCormick red food color for 40 min then 24 h later received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray; yellow and blue food color-aerosolized broilers (YBA;  $n = 9$ ;  $3,193 \pm 107$  g of BW) inhaled an aerosolized 1:1 mixture of McCormick yellow and blue food color for 40 min then 24 h later received 1 mL of 1 mg/mL of LPS via intratracheal aerosol spray; and propylene glycol-aerosolized broilers (PGA;  $n = 9$ ;  $3,496 \pm 126$  g of BW) inhaled aerosolized

PG (Sigma Chemical Co., 99.5% purity) for 40 min then 24 h later received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray. The PAP was recorded as described for the pilot PAP studies.

**Experiment 2.** Male broilers from a genetic line maintained on the University of Arkansas Poultry Research Farm were wing-banded and reared on previously used wood shaving litter in an open-sided poultry house equipped with Plasson drinkers. Birds from 35 to 53 d old were randomly assigned to 1 of 4 groups: nonaerosolized-saline broilers (NA-S;  $n = 9$ ;  $1,525 \pm 87$  g of BW) were not previously exposed to aerosol inhalation and received 1 mL of 0.9% NaCl via intratracheal aerosol spray; nonaerosolized-LPS broilers (NA-LPS;  $n = 10$ ;  $1,350 \pm 65$  g of BW) were not previously exposed to aerosol inhalation and received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray; nonaerosolized-LPS-i.v. broilers (NA-LPS i.v.;  $n = 10$ ;  $1,434 \pm 74$  g of BW) were not previously exposed to aerosol inhalation and were injected i.v. with 1 mL of 2 mg/mL of LPS; red food color-aerosolized broilers (RA;  $n = 10$ ;  $1,652 \pm 60$  g of BW) inhaled aerosolized red food color for 40 min then 24 h later received 1 mL of 1 mg/mL of LPS via intratracheal aerosol spray. The PAP was recorded as described for the pilot PAP studies.

**Experiment 3.** Male broilers from a different hatch of the same line that had been used in experiment 2 were wing-banded and reared on clean wood shavings litter in environmental chambers within the PERL. When they were 45 to 53 d old, the broilers were randomly assigned to 1 of 2 groups: nonaerosolized broilers (NA;  $n = 12$ ;  $2,611 \pm 117$  g of BW) were not previously exposed to aerosol inhalation and received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray, and Red Dye #3-aerosolized broilers (RD#3;  $n = 12$ ;  $2,647 \pm 57$  g of BW) inhaled aerosolized Red Dye #3 (Sigma Chemical Co.) mixed with PG (0.04 g of RD#3/mL of PG) for 40 min then 24 h later received 1 mL of 2 mg/mL of LPS via intratracheal aerosol spray. The PAP was recorded as described for the pilot PAP studies.

### Data Acquisition and Statistical Analyses

The Biopac MP 100 data acquisition system continuously recorded PAP (mmHg) during 2 control intervals of 2 min each (CTL1, CTL2) and during 22 intervals of 2 min each after administration of LPS (sampling intervals 2 to 44). The PAP data were averaged electronically within each of the 2-min intervals. The protocol used for data averaging accommodates the influences of pulse pressure and respiratory cycles on PAP (Wideman et al., 1996). Individual birds were considered to be the experimental unit within each treatment group ( $n$  = number of birds per treatment group). Data from each sampling interval were pooled within each treatment. Pooled data from CTL2 were compared against the following 22 intervals within treatments using 1-way repeated-measures ANOVA by time, and pooled data from each sample interval were compared among treatments using 1-way ANOVA by group (Jandel Scientific, 1994). Means were separated

by the Tukey test when the *F*-test from the 1-way ANOVA was declared significant ( $P < 0.05$ ).

## RESULTS

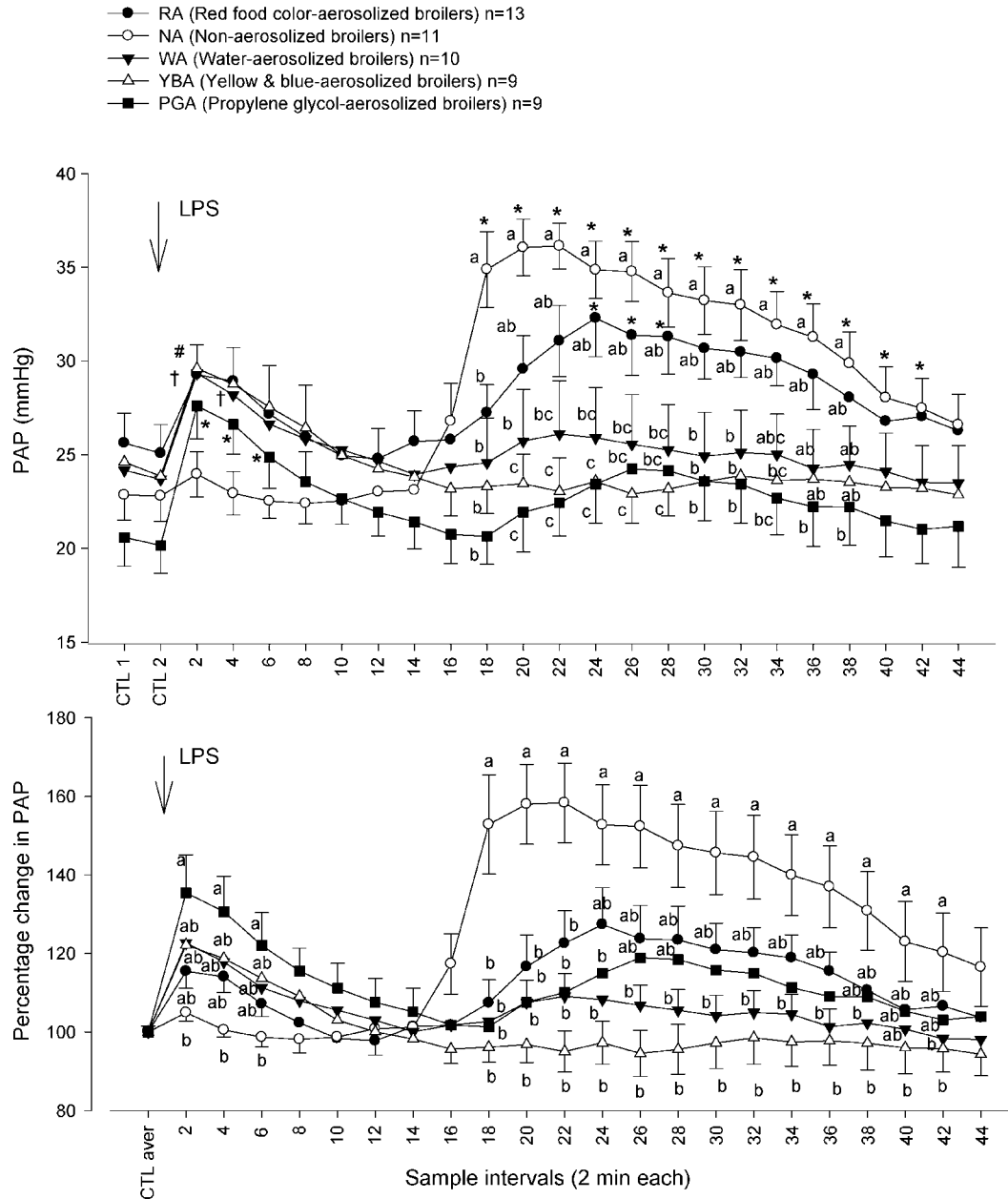
### Experiment 1

Absolute PAP values during control sample intervals (CTL1 and CTL2) averaged approximately 22.5, 23.7, 25.5, 24, and 20.5 mmHg for the NA, WA, RA, YBA, and PGA groups, respectively (Figure 1, upper panel). None of the PAP values differed significantly among the groups during sample intervals CTL1 to 16. Intravenous LPS administration caused the PAP in the NA group to increase above the initial control values of this group by sample interval 18, and the PAP remained elevated in the NA group until sample interval 44. All groups receiving the intratracheal LPS spray tended to exhibit an increase in PAP during the ensuing 2 min (sample interval 2) coinciding with a noticeable stress response caused by inserting the cannula and introducing 1 mL of aerosol into the trachea. The NA group received LPS via an i.v. injection and exhibited no comparable increase in PAP during sample interval 2. Within 4, 6, and 6 min, the PAP values for the YBA, WA, and PGA groups returned to levels that did not differ from their respective initial control values. After sample interval 8 only, the PAP values of the RA group again increased above the initial control level (sample intervals 24 to 28). Moreover, the PAP values for the RA group were not lower than those of the NA group throughout sample intervals 20 to 38 (Figure 1, upper panel). To normalize these responses for initial numerical differences in control PAP values, all data were recalculated as the percentage change in PAP compared with the average control PAP values (CTL1 and CTL2) as shown in the lower panel of Figure 1. During sample intervals 18 to 22, the NA group exhibited a dramatically higher percentage increase in PAP attributable the i.v. LPS injection, when compared with the percentage increase in PAP by all groups that received the intratracheal LPS aerosol, regardless of aerosol pretreatment. The values for the RA and PG groups increased by sample intervals 24 and 26, respectively, to peak levels that were not significantly lower than the contemporaneous values for the NA group. In contrast, the percentage change in PAP values for the WA and YBA groups remained lower than those of the NA group until the end of the experiment, with the exception of sample interval 40 for the WA group (Figure 1, lower panel).

### Experiment 2

The absolute PAP values during control sample intervals (CTL1 and CTL2) fell within a narrow range (18.5 to 20 mmHg) for all groups (Figure 2). All groups that received an intratracheal spray of saline (NA-S) or LPS (NA-LPS and RA) exhibited acute increases in their PAP values within 2 min (sample interval 2), whereas no increase in PAP was observed in the group that received LPS via

## Experiment 1

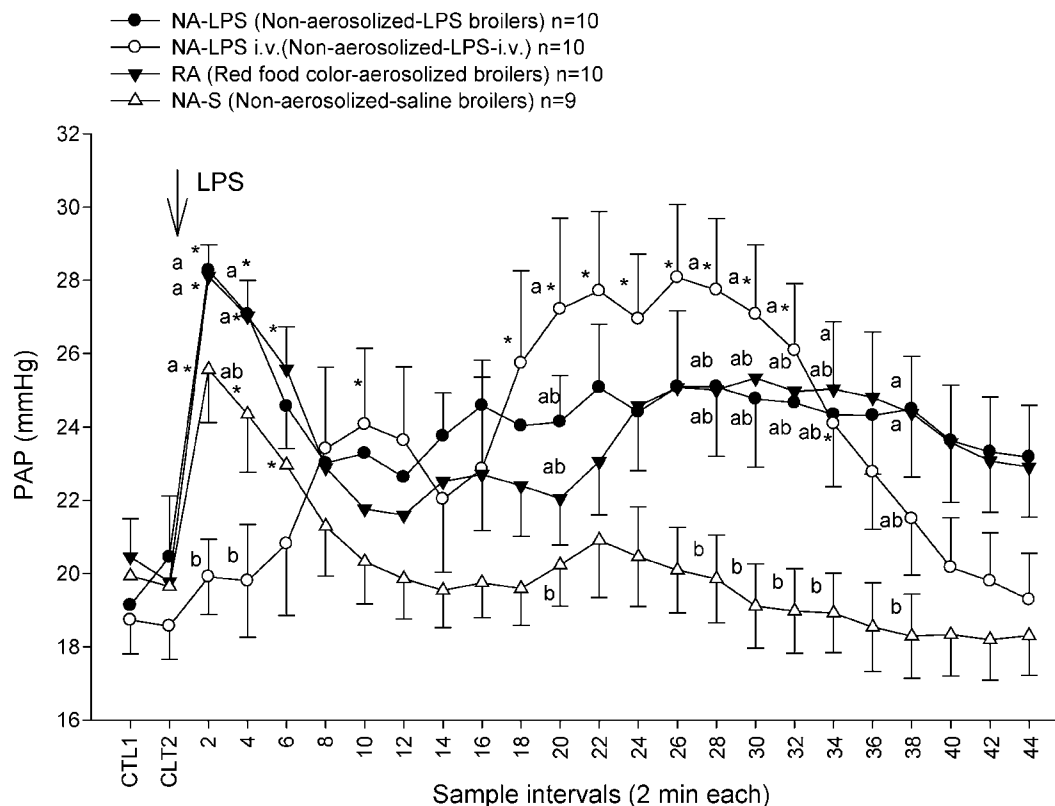


**Figure 1.** Upper panel: pulmonary arterial pressure (PAP; mean  $\pm$  SEM) of male broilers reared in environmental chambers in experiment 1. The PAP was measured in non-aerosolized broilers after an i.v. injection of 1 mL of 2 mg/mL of lipopolysaccharide (LPS; NA;  $n = 11$ ) and in propylene glycol-aerosolized broilers (PGA;  $n = 9$ ), red food color-aerosolized broilers (RA;  $n = 13$ ), yellow and blue-aerosolized broilers (YBA;  $n = 9$ ), and water-aerosolized broilers (WA;  $n = 10$ ) after an intratracheal spray of 1 mL of 2 mg of LPS. Data were averaged electronically during representative sample intervals before (control sample intervals CTL 1 and 2) and after the LPS administration (sample intervals 2 to 44). Asterisks (\*) for the NA and RA, crosses (†) for the YBA, and number symbol (#) for the WA groups denote values higher than the pre-LPS values of the respective group (sample intervals CTL 1 and 2). Lower panel: PAP of the NA, PGA, RA, YBA, and WA groups expressed as a percentage of change in PAP compared with their respective pre-LPS values (sample intervals CTL 1 and 2). Different letters (a,b,c) designate group means that differed within a sample interval ( $P \leq 0.05$ ). Arrows indicate LPS administration. aver = average.

an i.v. injection (NA-LPS i.v.) during the same sample interval. The initial response to the intratracheal spray had subsided by sample interval 8, and the groups did not differ again until sample interval 20 when the PAP of the NA-LPS i.v. group was higher than the PAP of the NA-S group but not higher than the values of the NA-LPS or RA groups. When compared with initial control

values CTL1 and CTL2, the groups that received LPS i.v. (NA-LPS i.v.) or as an intratracheal aerosol spray (NA-LPS, RA) tended to exhibit sustained pulmonary hypertensive responses to LPS (sample intervals 20 to 38), whereas the PAP gradually declined in the NA-S group. After sample interval 40, the PAP did not differ between groups (Figure 2).

## Experiment 2



**Figure 2.** Pulmonary arterial pressure (PAP; mean  $\pm$  SEM) of male broilers from a genetic line maintained on the University of Arkansas Poultry Research Farm reared on previously used floor litter in an open-sided poultry house in experiment 2. The PAP was measured in non-aerosolized broilers receiving 1 mL of NaCl via intratracheal spray (NA-S;  $n = 9$ ), non-aerosolized broilers after i.v. (NA-LPS i.v.;  $n = 10$ ) or intratracheal (NA-LPS;  $n = 10$ ) administration of 1 mL of 2 mg/mL of lipopolysaccharide (LPS), and red food color-aerosolized broilers (RA;  $n = 13$ ) after intratracheal spray of 1 mL of 2 mg/mL of LPS. Data were averaged electronically during representative sample intervals before (control, sample intervals CTL 1 and 2) and after the LPS administration (sample intervals 2 to 44). Asterisks (\*) denote values higher than the pre-LPS values of the respective group (sample intervals CTL 1 and 2). Different letters (a,b) designate group means that differed within a sample interval ( $P \leq 0.05$ ). Arrow indicates LPS administration.

## Experiment 3

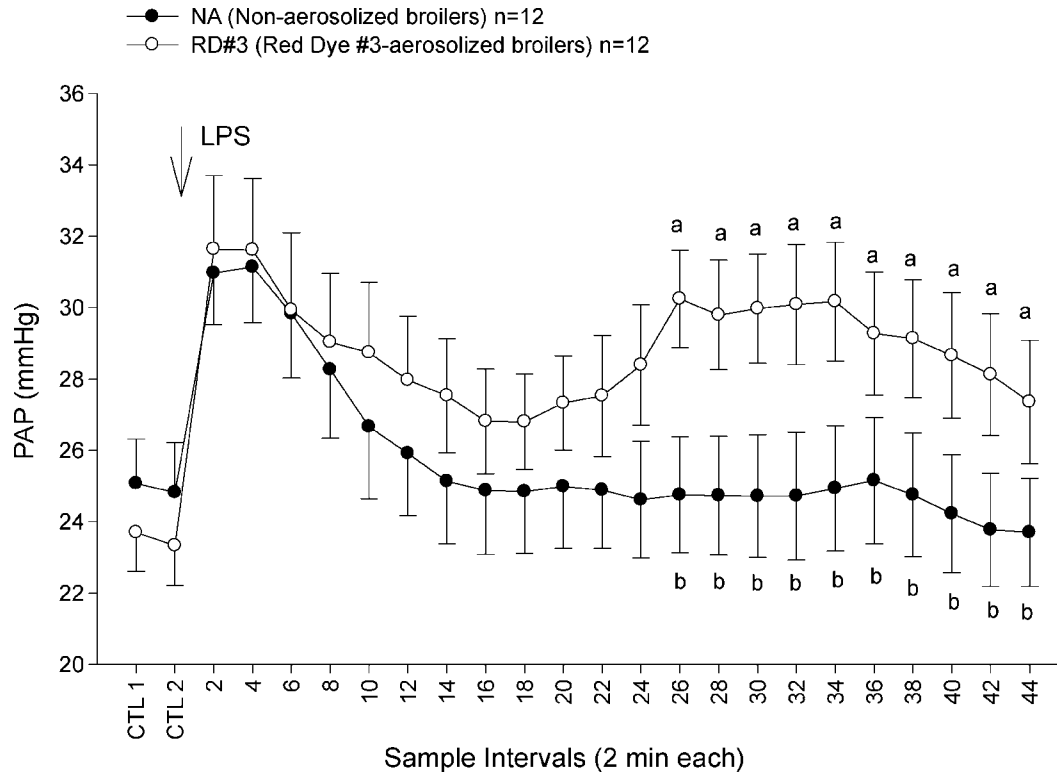
Both NA and RD#3 groups increased their PAP within 2 min after the intratracheal LPS aerosol spray (Figure 3). The PAP did not differ between groups until sample interval 26 when the RD#3 group had higher PAP values than the NA group. From sample interval 26 and throughout the rest of the experiment, the PAP values of the RD#3 group remained higher than the PAP values of the NA group (Figure 3). Variation in the PAP responses among individual broilers is shown in Figure 4. The intratracheal aerosol spray caused the PAP to increase by at least 4 mmHg between sample intervals 16 to 38 in 3 of 12 broilers in the NA group (Figure 4, upper panel) and 11 of 12 broilers in the RD#3 group (Figure 4, lower panel).

## DISCUSSION

The PAH responses to LPS clearly were consistently higher using the i.v. rather than the aerosol route of administration. When injected i.v., LPS molecules within the blood vessels rapidly interact with receptors on the surface of immune cells to initiate signaling cascades lead-

ing to the release of proinflammatory mediators and vasoactive factors (Wideman et al. 2001, 2004). In contrast, LPS administered via the airways must overcome multiple mucosal defenses before proinflammatory responses can be activated. The respiratory tract must guarantee effective, ongoing gas exchange while providing appropriate defense against pathogens. Mucosal defenses must provide effective surveillance and discrimination between threatening and nonthreatening agents (Ramnik and Podolsky, 2000; Granucci and Ricciardi-Castagnoli, 2003). While larger airborne particles are trapped in the nasal cavities and trachea, smaller respirable particles averaging 1.1  $\mu\text{m}$  are able to reach the lung gas exchange parenchyma and abdominal air sacs (Fulton et al., 1990). Respirable particles can be heavily contaminated with a wide range of immunogenic substances including pathogens and toxins (Bakutis et al., 2004). Sustaining ongoing vigorous immune responses against these substances may incur more damage than protection to the lungs of the host (Ewaschuk and Dieleman, 2006). Accordingly, pulmonary mucosal defenses appear to be designed to bind, inactivate, or discriminate among inhaled antigens, thereby applying a pattern of tolerance of nonthreatening

## Experiment 3



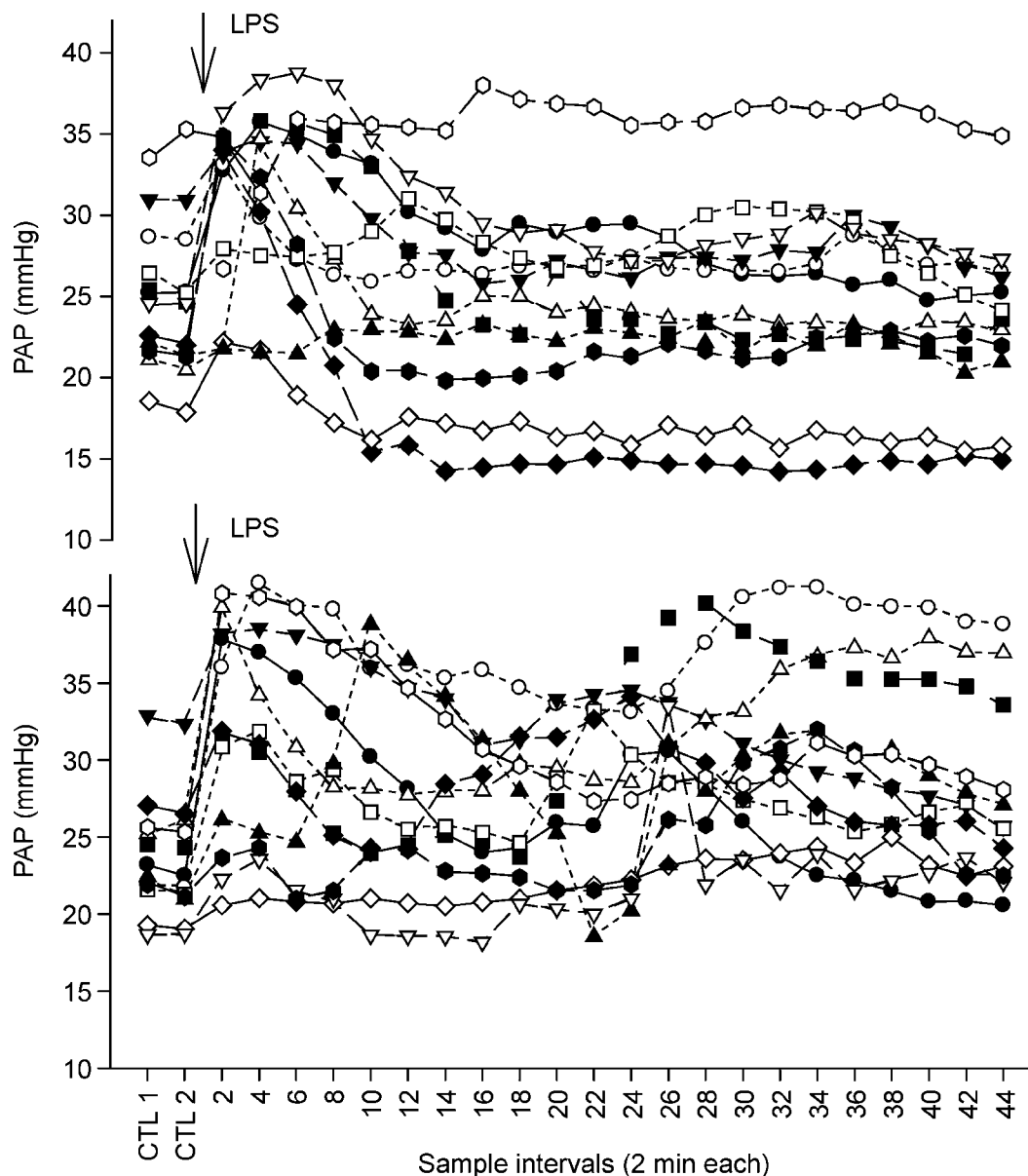
**Figure 3.** Pulmonary arterial pressure (PAP; mean  $\pm$  SEM) in male broilers reared in environmental chambers in experiment 3. Non-aerosolized (NA;  $n = 12$ ), and Red Dye #3 plus propylene glycol-aerosolized broilers (RD#3;  $n = 12$ ) received an intratracheal spray of 1 mL of 2 mg/mL of lipopolysaccharide (LPS). Data were averaged electronically during representative sample intervals before (control, sample intervals CTL 1 and 2) and after LPS administration (sample intervals 2 to 44). Different letters (a,b) designate group means that differed within a sample interval ( $P \leq 0.05$ ). Arrow indicates LPS administration.

agents to minimize counterproductive chronic inflammatory responses within the gas exchange parenchyma.

Surfactant proteins have been proposed to modulate immune responses in the airways (Crouch, 1998; Borron et al., 2000). Mice deficient in surfactant protein A (SP-A) are prone to develop a more robust immune response against LPS than are mice that have normal levels of SP-A. For example, SP-A attenuates LPS-induced production of tumor necrosis factor  $\alpha$  and macrophage inflammatory protein-2 in bronchoalveolar fluid in vivo (Borron et al., 2000). In addition, SP-A reduces the proliferative response of T cells to mitogens, a process thought to be mediated by the binding of SP-A to a 210-kDa receptor on lymphocyte and macrophage membranes (Borron et al., 1998). In mammals, SP-A is extensively present in upper and lower airways including alveoli that are known to be populated with alveolar macrophages. In birds, macrophages normally are not present within the air capillaries where gas exchange occurs, but macrophages have been detected in the atria and infundibula of the parabronchi, as well as in the larger conducting airways (Maina and Cowley, 1998; Nganpiep and Maina, 2002). Coincidentally, SP-A has not been found in the air capillaries or parabronchi (tertiary bronchi), but SP-A is localized in subsets of epithelial cells of the secondary bronchi (Zeng et al., 1998; Johnston et al., 2000) where a regulatory effect on macro-

phages and lymphocytes may be exerted. Evidently, in the present study, the mucosal defense mechanisms of broilers reared within environmental chambers effectively prevented aerosolized LPS from triggering counterproductive responses such as behavioral depression, reduced BW gain, and PAH except when the airways of the birds had been suitably primed or sensitized.

Consistently throughout the 3 experiments, all groups receiving the intratracheal LPS spray tended to exhibit an increase in PAP during the ensuing 2 min (sample interval 2) coinciding with a noticeable stress response caused by inserting the cannula and introducing 1 mL of aerosol into the trachea. Epinephrine is known to trigger transient pulmonary vasoconstriction and pulmonary hypertension in broilers (Wideman, 1999; Lorenzoni, 2006). Experiments 1 and 3 demonstrated that pretreating broilers with aerosolized red food color and its key ingredients, Red Dye #3 and PG, sensitized or primed the respiratory system so that the broilers more consistently exhibited a pulmonary hypertensive response to a subsequent intratracheal LPS aerosol spray. Pretreatment with aerosolized water or nonred food colors failed to sensitize the broilers in experiment 1. Ingestion of Red Dye #3 has been implicated in human cases of bronchoconstriction, urticaria, and rhinitis, and i.v. injections of the carrier vehicle PG have been associated with dermatitis, phlebi-



**Figure 4.** Individual responses of pulmonary arterial pressure (PAP; mean) in male broilers reared in environmental chambers in experiment 3. Upper panel: PAP in non-aerosolized (NA;  $n = 12$ ) broilers during representative sample intervals before (control, sample intervals CTL 1 and 2) and after an intratracheal spray of 1 mL of 2 mg/mL of lipopolysaccharide (LPS; sample intervals 2 to 44). Lower panel: PAP in Red Dye #3 plus propylene glycol-aerosolized broilers (RD#3;  $n = 12$ ) during representative sample intervals before (control, sample intervals CTL 1 and 2) and after an intratracheal spray of 1 mL of 2 mg/mL of LPS (sample intervals 2 to 44). Arrows indicate LPS administration.

tis, and increases in blood histamine levels (Fisherman and Cohen, 1973; Berlin, 1997; Doenicke et al., 1999). It is possible that Red Dye #3 and PG may interfere with the modulatory effect of surfactant proteins on the immune cells within the airways. Red Dye #3 and PG also may initiate a modest inflammatory response within the airways. Inflammation may induce production and secretion of cytokines, thereby overcoming the suppressor effect of surfactant proteins and stimulating the migration of immune cells from the interstitium into the airways. Leukocytes recruited into the airways would be directly accessible to respirable LPS, permitting an intimate interaction and thus more direct activation of the innate immune response.

In experiment 2, exposure to previously used floor litter within a curtain-sided house appeared to naturally prime or sensitize the airways of the broilers. This was the only experiment in which broilers that were not pretreated with aerosolized red food color or its key ingredients nevertheless consistently exhibited significant PAH after LPS was administered as an intratracheal spray. Perhaps a similar natural airway sensitization might be anticipated when broilers chronically inhale common airborne pollutants and gasses that normally are present in commercial poultry houses. Mechanisms by which the airways became sensitized to LPS in the present study remain to be determined. It has been demonstrated previously that intratracheal instillation of *Corynebacterium parvum* or *E.*

*coli* effectively increased the number of phagocytes collected by lung lavage within 24 h (Toth et al., 1987). Additionally, macrophages have been reported to migrate into air capillaries in a variety of infectious diseases, including toxoplasmosis, fatal viral hydropericardium syndrome, highly pathogenic infectious bursal disease, and highly pathogenic avian influenza (Howerth and Rodenroth, 1985; Abe et al., 1998; Nakamura et al., 2001). Inhaling low doses of toxins and pathogens, or primers such as Red Dye #3 and PG, may induce subclinical levels of lung inflammation and immigration of immune cells, thereby unbalancing the mechanisms of tolerance normally exhibited by the pulmonary mucosal immune system. Actually, oxidized glutathione (an index of tissue oxidative stress) and blood CO<sub>2</sub> (index of cardiopulmonary system performance) have been reported higher in birds reared on floor litter when compared with birds reared in clean environments (environmental chambers and stainless steel cages, respectively; Bottje et al., 1998; Wang et al., 2002b). Treatment with aerosolized Red Dye #3 and PG appears to constitute a nonpathogenic, effectively controllable experimental model for deducing the mechanisms by which air pollutants commonly present in commercial poultry houses can enhance pulmonary hypertensive responses of broilers to respirable LPS. In fact, hypertensive response to intratracheal LPS can be detected sporadically in nonaerosolized birds reared in environmental chambers (Figure 4, upper panel), and relative unresponsiveness also occurs in birds raised under commercial conditions. However, the proportion of responders is dramatically increased when birds are either reared under commercial conditions or are primed with aerosolized Red Dye #3 and PG (Figure 4, lower panel).

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# Differential expression of vasoactive mediators in microparticle-challenged lungs of chickens that differ in susceptibility to pulmonary arterial hypertension

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**Hamal KR, Wideman RF, Anthony NB, Erf GF.** Differential expression of vasoactive mediators in microparticle-challenged lungs of chickens that differ in susceptibility to pulmonary arterial hypertension. *Am J Physiol Regul Integr Comp Physiol* 298: R235–R242, 2010. First published November 11, 2009; doi:10.1152/ajpregu.00451.2009.—Pulmonary hypertension syndrome (PHS; ascites) in fast growing meat-type chickens (broilers) is characterized by the onset of idiopathic pulmonary arterial hypertension (IPAH) leading to right-sided congestive heart failure and terminal ascites. Intravenous microparticle (MP) injection is a tool used by poultry geneticists to screen for the broilers that are resistant (RES) or susceptible (SUS) to IPAH in a breeding population. MPs occlude pulmonary arterioles and initiate focal inflammation, causing local tissues and responding leukocytes to release vasoactive mediators such as serotonin (5-HT), endothelin-1 (ET-1), and nitric oxide (NO). RT-PCR was used to examine the differences between RES and SUS broilers in terms of gene expression of ET-1, ET receptor types A and B (ET<sub>A</sub> and ET<sub>B</sub>), the serotonin transporter (SERT), serotonin receptors (5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>2B</sub>), endothelial NO synthase (eNOS), and inducible NOS (iNOS) in the lungs of these broilers before (0 h) and after (2, 6, 12, 24, and 48 h) MP injection. In SUS broilers MP injection elicited higher ( $P < 0.05$ ) pulmonary expression of 5-HT<sub>1A</sub>, 5-HT<sub>2B</sub>, and ET-1, which promote vasoconstriction and proliferation of pulmonary arterial smooth muscle cells (PASMC). In RES broilers the MP injection elicited higher expression of eNOS, iNOS, and ET<sub>B</sub>, which promote vasodilation and inhibit PASMC proliferation. These observations support the hypothesis that the resistance of broiler chickens to IPAH may be due to the higher expression of vasoactive mediators that favor enhanced vasodilation and attenuated vasoconstriction during MP injection challenges to the pulmonary vasculature.

ascites; pulmonary arterial hypertension; endothelin-1; nitric oxide; serotonin

PULMONARY HYPERTENSION SYNDROME (PHS; ascites) in fast-growing, meat-type chickens (broilers) is characterized by the onset of idiopathic pulmonary arterial hypertension (IPAH) leading to right-sided congestive heart failure and fluid accumulation in the abdominal cavity (ascites). Intravenous microparticle (MP) injection is a patented method (US patent no. 6,720,473) used by poultry geneticists to screen for the resistant (RES) and susceptible (SUS) broilers in a breeding population. Microparticles occlude precapillary arterioles and increase pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP) in proportion to the numbers of MP injected (6, 55). The entrapped MP rapidly initiate inflammation and stimulate the local tissues and leukocytes to release

vasoactive mediators, such as endothelin-1, serotonin (5-HT), and nitric oxide that can alter the PVR either by constricting (serotonin, endothelin-1) or dilating (nitric oxide) nearby blood vessels (6, 7, 18, 54, 57, 62).

Endothelin-1 has an important role in the pathogenesis of IPAH (15, 32). It is produced by endothelial cells (23, 24) and acts by binding to two endothelin receptors, ET<sub>A</sub> and ET<sub>B</sub> (19, 40). The ET<sub>A</sub> receptors are expressed exclusively on pulmonary arterial smooth muscle cells (PASMC) (21), whereas ET<sub>B</sub> receptors are expressed predominantly on endothelial cells and at lower levels on PASMC (36). Binding of endothelin-1 to ET<sub>A</sub> and ET<sub>B</sub> receptors on the PASMC leads to vasoconstriction (38) and PASMC proliferation (9, 43). Binding of endothelin-1 to ET<sub>B</sub> receptors leads to vasodilation through the release of nitric oxide and prostacyclin (20). In chickens, endothelin-1 produced dose-dependent contractile responses in pulmonary artery rings, and these responses were modulated by nitric oxide (29, 48). The ET<sub>A</sub> receptor antagonist BQ123 attenuated the development of pulmonary hypertension and right ventricular hypertrophy in broilers chronically exposed to low ambient temperatures (63). The lungs of broilers with advanced IPAH had higher expression of endothelin-1 but lower expression of ET<sub>A</sub> when compared with the healthy flock mates (17).

Serotonin and the serotonin transporter contribute to the pathogenesis of human IPAH (14, 25) and PAH induced by hypoxia (12) and by serotonergic appetite suppressant drugs (1, 13). Serotonin causes vasoconstriction by interacting with its receptors 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2B</sub>, which are expressed on PASMC (8, 28, 47, 51). A role for the 5-HT<sub>1A</sub> receptor subtype in IPAH pathogenesis had not been demonstrated. Serotonin transporter is abundantly expressed in the lung and is primarily located on PASMC (35). Serotonin transported into the intracellular compartment of PASMC causes smooth muscle cell proliferation and vascular remodeling (11, 26). In broilers, MP entrapment within the pulmonary vasculature stimulates thrombocytes to release serotonin, resulting in potent pulmonary vasoconstriction and pulmonary hypertension, which can be prevented by pretreatment with methiothepin, a nonselective 5-HT<sub>1/2</sub> receptor antagonist (4, 5, 6, 7). Methiothepin pretreatment also reduced by  $\geq 60\%$  the postinjection mortality caused by injecting MP into SUS broilers, demonstrating a central role for serotonin during the MP challenge (5, 6, 7).

Nitric oxide is synthesized in broiler lungs by endothelial nitric oxide synthase (eNOS) and inducible NOS (iNOS) expressed by activated macrophages (18, 22, 33, 61). The competitive inhibitor *N*<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) blocks NOS, acutely increases the PVR and PAP, and doubles

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the mortality caused by MP injections in broilers (50, 52, 57, 58, 60, 61, 62). Repeated intraperitoneal injections of L-NAME caused PAH and ascites in broilers (16), whereas dietary supplementation with L-arginine elevated plasma nitric oxide levels, attenuated pulmonary arteriole remodeling, induced vasodilation, and reduced IPAH-related mortality in broilers (44, 52, 53). Nitric oxide plays a key role in broiler lungs as the primary flow-dependent vasodilator and modulator of vasoconstriction (18, 57, 61, 62). When compared with healthy flock mates, broilers with advanced IPAH had either reduced eNOS expression in pulmonary arterioles (30, 31) or unaltered pulmonary eNOS and iNOS expression (46). Previously, we demonstrated that intravenous MP injections elicit increased pulmonary iNOS expression in broilers from SUS and RES genetic lines and that at 24 h postinjection RES broilers had higher pulmonary iNOS expression than SUS broilers (18). Expression of eNOS in response to MP injection was not evaluated in that experiment.

In this study, MP were injected intravenously into clinically healthy broilers from the RES and SUS lines. Clinically healthy SUS broilers typically have higher PVR and PAP and are more susceptible to MP-induced mortality than the RES broilers (2, 6, 56). Our objective was to examine the expression of genes for key vasoactive mediators, such as endothelin-1 and its receptors, serotonin receptors and transporter, and NOS before and for up to 48 h after the MP injection. We hypothesized that resistance to IPAH in broilers may be conferred by higher expression of those vasoactive mediators that favor enhanced vasodilation in combination with attenuated vasoconstriction during hypertensive challenges to the pulmonary vasculature.

## MATERIALS AND METHODS

### Broiler Management

All animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee. The RES and SUS broiler lines used for this study were developed by divergent selection based on rearing them in a hypobaric chamber. At the time of this study the lines were at the ninth generation of their selection and exhibited ascites mortalities of 7.5% (RES line) and 75% (SUS line) when reared under hypobaric conditions (37).

Fifty male chicks per line were reared on fresh wood shavings in environmental chambers (8-m<sup>2</sup> floor space). Chicks were brooded at 33.2°C on days 1 to 3, at 31.1°C on days 4 to 6, at 29.4°C on days 7 to 10, at 25.5°C on days 11 to 14, and at 23.9°C, day 15 onwards. Feed and water were provided ad libitum, and light schedules were 24 h/day for days 1 to 4 and 16:8-h light-dark from day 5 onward.

### Microparticle Injection, Lung Collection, and Quantification of Microparticles

Broilers (4 wk old) were injected with MP as described previously (49, 55). Briefly, CM-32 ion exchange cellulose (Fisher Scientific, St. Louis, MO) was suspended at the rate of 0.02 g/ml in heparinized saline. This suspension (0.35 ml/broiler) was injected via the wing vein using a 22-gauge needle attached to 1-ml tuberculin syringe (Becton Dickinson, Franklin Lake, NJ).

The portion of the right lung between the first and second anterior rib indentation (costal sulcus) was collected from six broilers per line at 0 (uninjected control lung), 2, 6, 12, 24, and 48 h post-MP injection. The lungs were sliced into 5–6 pieces, immersed in 3 ml of RNAlater RNA preservation buffer (Qiagen, Valencia, CA), stored at 4°C overnight and at –20°C until RNA extraction. An adjacent segment

from each lung was immersed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. To ensure that each lung had entrapped a similar number of MP, the number of MP in each lung section was quantified. For this, hematoxylin and eosin-stained lung sections (6/line/time point) were examined microscopically for MP by using a computerized image analysis system comprising a Cool SNAP cf digital camera (Image Processing Solutions, North Reading, MA) and Image Pro Plus software (Media Cybernetics, Silver Spring, MD). For each section, four randomly selected microscopic fields were evaluated at ×10 magnification by using an Olympus BX50 microscope (Olympus America, Center Valley, PA). The number of MP in the each lung section was calculated by counting the MP in four microscopic fields per section and averaging the number of MP per microscopic field. At each time point, the average number of MP was similar ( $P > 0.05$ ) in both lines. The average number of MP per microscopic field per lung section was 5.5.

### Real-Time RT-PCR

**RNA isolation.** RNA was isolated from the RNAlater preserved lung tissues (91 to 99 µg per sample) using the Aurum total RNA fatty and fibrous tissue kit (Bio-Rad, Hercules, CA) and following the spin format protocol with slight modification. To remove contaminating DNA, if any, an additional DNA digestion step was performed. The RNA samples were aliquoted into four subsamples and stored at –80°C until analysis.

**Assessment of quality and quantity of RNA.** The quality and quantity of RNA were examined using an Experion automated electrophoresis system and the Experion RNA StdSens analysis kit (Bio-Rad) following the manufacturer's protocol with some modifications. Automated electrophoresis provided the RNA concentration for each sample, a virtual gel, and an electropherogram showing the 18S and 28S band peak. Intact and crisp 18S and 28S bands indicate good quality of RNA (virtual gel not shown).

**Reverse transcription.** RNA (2.5 µg/sample) was reverse transcribed to cDNA by using Taqman reverse transcription reagents (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol in a Biometra personal cycler (Biometra, Göttingen, Germany). Briefly, 40 µl of each reaction mixture contained 4 µl of 10× RT buffer, 1.6 µl of 25× dNTP mix (100 mM), 4 µl of 10× RT random primers, 2 µl of MultiScribe reverse transcriptase (50 U/ml), and 2.5 µg total RNA with nuclease-free H<sub>2</sub>O added to bring the volume to 40 µl. The incubation steps used were: one cycle of 25°C for 10 min, 37°C for 120 min, and 85°C for 5 s. After cDNA synthesis, the cDNA samples were aliquoted into four subsamples of 10 µl each and stored at –80°C until analysis.

**Quantification of relative gene expression of vasoactive mediators.** Real-time PCR was performed using TaqMan Universal PCR master mix in an ABI PRISM 7300 sequence detection system (Applied Biosystems). The PCR was performed in a reaction volume of 25 µl containing the reagents at the following final concentrations: 1× TaqMan Universal PCR Master Mix (2×), forward primer 200 nM, reverse primer 200 nM, probe 100 nM, and 1 µl of cDNA sample. The cycling profiles used were 1 cycle at 50°C for 2 min, 95°C for 10 min, and 40 cycles (95°C for 15 s, and 60°C for 60 s). Previously published primers and probes for 28S and chicken iNOS were used for PCR (41). The primers and probes for the remaining target genes were designed by the author using primers express software version 2.0 (Applied Biosystems). The sequences for the primers and probes used are listed in Table 1. In each plate, a no-template control (no cDNA, master mix only), a calibrator sample, cDNA samples, and endogenous control (28S) were included. Endogenous controls (28S) were analyzed in duplicate, and the target genes were analyzed in triplicate. The calibrator sample was cDNA from the 0-h lung samples that had not been injected with MP. The relative gene expression was quantified by the  $\Delta\Delta C_t$  method. The fold change in gene expression was calculated by comparing the gene expression of the sample with the

Table 1. Primers and probe sequences used for real-time RT-PCR

Target	Primer or Probe*	Sequences	Exon Boundary	GenBank Accession No.
28S†	Forward	5'-GGCGAAGCCAGAGGAACT-3'	not known	X59733
	Reverse	5'-GACGACCGATTTCACGTC-3'		
	Probe	5'-AGGACCGCTACGGACCTCCACCA-3'		
iNOS†	Forward	5'-TTGGAAACCAAAGTGTGAATATCTTG-3'	Exon 27	U46504
	Reverse	5'-CCCTGGCCATGCGTACAT-3'		
	Probe	5'-TCCACAGACATACAGATGCCCTTCTCTT-3'		
eNOS‡	Forward	5'-GGCAGTATGGTGTGGTGT-3'	Exon 2	XM_001236739
	Reverse	5'-TCCATCTCACGCCGGTAGAT-3'		
	Probe	5'-CCGATCTCCGCGCTGGACCA-3'		
ET-1‡	Forward	5'-CGTGATTTCTGCCACCTGGAT-3'	2/3	XM_418943
	Reverse	5'-AGGGCCTCCAAGACCATAGG-3'		
	Probe	5'-CATCTGGATCAACACCCCGAGAAGA-3'		
ET <sub>A</sub> ‡	Forward	5'-ACAGAAGGAACAGCAACTTGAGAA-3'	5/6	NM_204119
	Reverse	5'-GGGAACCAGCAAAGAGCAAA-3'		
	Probe	5'-CTCTCAGTGAACACCTTAAGCAGCGTGA-3'		
ET <sub>B</sub> ‡	Forward	5'-CATCATCGACATCCCCATCA-3'	1/2	XM_417001
	Reverse	5'-CACTAATTACACATTTGACACCAA-3'		
	Probe	5'-TCTACAAGCTACTTGCAGAGGACTGGCCC-3'		
SERT‡	Forward	5'-CTATTGGCTTATGCCAGCTACAAC-3'	Exon 6	NM_213572
	Reverse	5'-ACGAAGCTGGTCAGGCAGTT-3'		
	Probe	5'-AATTCCATAACAACCTGCTACCAAGACGCC-3'		
5-HT <sub>1A</sub> ‡	Forward	5'-CGAGGACCGCTCAAATCCT-3'	Exon 1	XM_429136
	Reverse	5'-GCGCCGAAGGTGGAGTAGA-3'		
	Probe	5'-ACCATCAGCAAGGACCGGGTACAG-3'		
5-HT <sub>2A</sub> ‡	Forward	5'-GGTTTAAGTCAAGAACAAGGCTTTT-3'	2/3	XM_425628
	Reverse	5'-CCTTTCTTAAACACTTTGCAGTCATT-3'		
	Probe	5'-TTGGACCATATCAGTTGGTATCTCCATGCC-3'		
5-HT <sub>1B</sub> ‡	Forward	5'-CATTTGGAATGCGATGTGTTCT-3'	1/2	XM_419875
	Reverse	5'-TTCAAGCAGTGGGTCTTACATA-3'		
	Probe	5'-TGCCGATGCCACAGAATCAAGTTG-3'		
5-HT <sub>2B</sub> ‡	Forward	5'-GTCGACAGTGTCCACAGTATTTTCAG-3'	not known	AF217255.1
	Reverse	5'-TCAGCATGGCCACCTTTCT-3'		
	Probe	5'-CGATGCCACACCTGCCCTGCTCA-3'		

iNOS, inducible nitric oxide synthase; eNOS, endothelial NOS; ET-1, endothelin-1; ET<sub>A</sub> and ET<sub>B</sub>, ET receptor types A and B; SERT, serotonin transporter. \*Probes had 6-carboxyfluorescein (FAM) at the 5' end and 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. †Previously published primers and probes (41); ‡primers and probes designed by the author.

expression of the calibrator sample from a broiler that was not MP injected.

#### Statistical Analysis

Using JMP Statistical Software (version 7.0.1; SAS Institute, Cary, NC), one-way ANOVA was carried out to determine differences in the fold change of target genes in the lungs of broilers from RES and SUS lines at a given time point and between samples collected at the various time points within each line. Differences among the group means were determined by Fisher's least significant difference multiple mean comparisons test. Data were expressed as means  $\pm$  SE, and the differences were considered significant at  $P \leq 0.05$ .

## RESULTS

### Endothelin-1 and Its Receptors, ET<sub>A</sub> and ET<sub>B</sub>

Endothelin-1 expression increased in the lungs of both RES and SUS broilers by 2 h postinjection (3.3-fold increase in RES vs. 7.2-fold increase in SUS, Fig. 1A) and remained elevated thereafter. Compared with the lungs from RES broilers, the lungs from SUS broilers had higher expression of endothelin-1 at 2 h ( $P = 0.0016$ ), 6 h ( $P = 0.0024$ ), and 12 h ( $P = 0.0119$ ) postinjection (Fig. 1A).

In both lines, ET<sub>A</sub> receptor expression more than doubled by 6 h postinjection and remained elevated throughout the 48-h period (Fig. 1B). The SUS and RES lungs did not differ in

expression of ET<sub>A</sub> at any of the time points examined (Fig. 1B). In both lines, ET<sub>B</sub> receptor expression almost tripled by 2 h (Fig. 1C), remained at this level until 6 h, and then declined at the 12-h time point. Thereafter, ET<sub>B</sub> expression increased again in RES lungs reaching higher expression levels of ET<sub>B</sub> at 24 ( $P < 0.0001$ ) and 48 h ( $P < 0.0001$ ) postinjection when compared with the SUS lungs (Fig. 1C).

### Serotonin Receptors and Transporter

Expression of the 5-HT<sub>1A</sub> receptor increased within 6 h after MP injection in RES lungs (3.5-fold increase) or within 2 h postinjection in SUS lungs (4.9-fold increase) and then remained elevated throughout subsequent time points in both lines (Fig. 2A). Lungs from the SUS broilers had higher expression of 5-HT<sub>1A</sub> at 2 h ( $P = 0.010$ ), 6 h ( $P = 0.0009$ ), and 12 h ( $P = 0.0062$ ) postinjection when compared with the lungs from RES broilers (Fig. 2A). 5-HT<sub>2A</sub> expression increased within 2 h postinjection in the RES lungs (2.6-fold increase) or by 6 h in the SUS lungs (3.2-fold increase) and remained elevated throughout subsequent time points in both lines. Lungs from the two lines did not differ in 5-HT<sub>2A</sub> expression at any of the time points (Fig. 2B). Expression of 5-HT<sub>1B</sub> receptors increased within 6 h postinjection in the lungs of RES broilers (2.4-fold increase) or within 12 h in SUS lungs (3.0-fold increase) and remained elevated at all subsequent

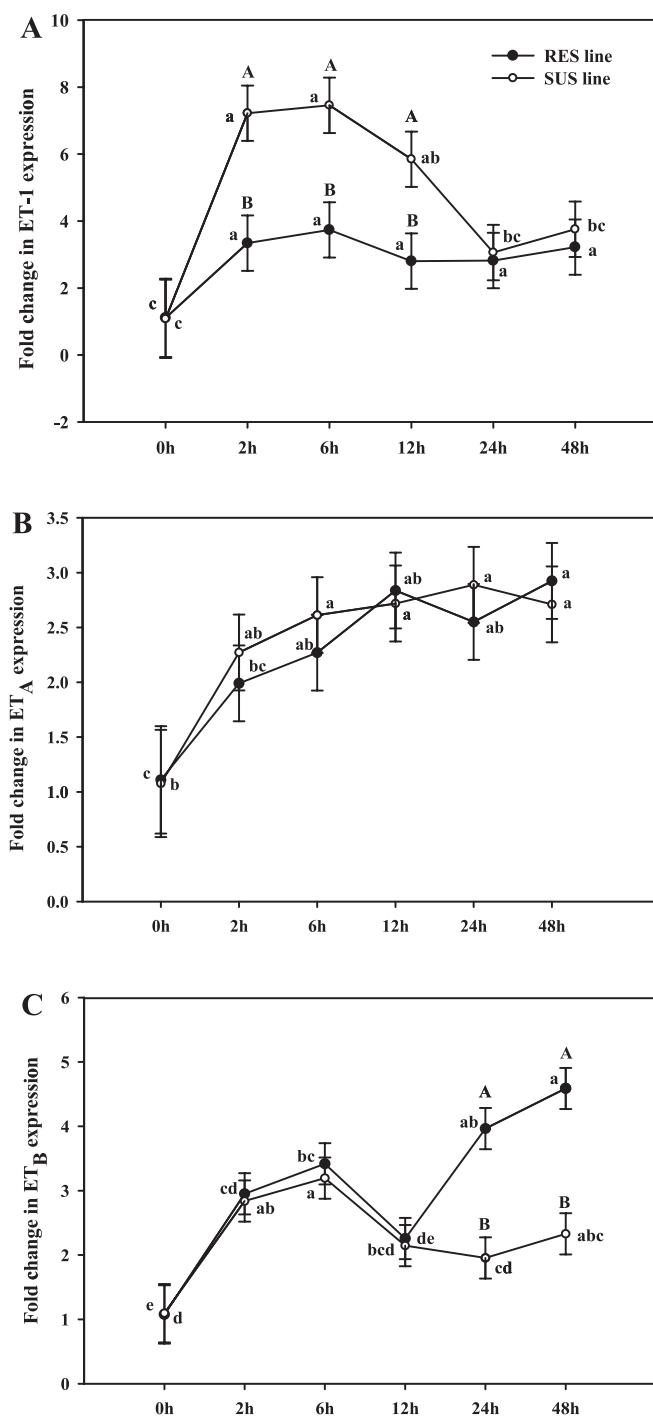


Fig. 1. Fold change in gene expression of endothelin-1 (ET-1; A), endothelin receptors type A (ET<sub>A</sub>; B) and type B (ET<sub>B</sub>; C) in lungs of broilers from the ascites-resistant (RES) and -susceptible (SUS) lines before (0 h) and after (2, 6, 12, 24, and 48 h) intravenous microparticle (MP) injection. Relative gene expression was quantified by real-time RT-PCR using an Applied Biosystems 7300 sequence detection system. The fold change in gene expression was computed by the  $\Delta\Delta C_t$  method, where the MP uninjected cDNA sample was used as the calibrator and 28S cDNA served as an endogenous control. Data are expressed as means  $\pm$  SE ( $n = 6$  per line and time point). Letters A and B indicate line differences between each time point; letters a, b, c, and d indicate differences among the time points within each line. For each analysis, means without a common letter are different ( $P \leq 0.05$ ).

time points in both lines. Lungs from both lines did not differ in 5-HT<sub>1B</sub> expression at any of the time points (Fig. 2C). Expression of 5-HT<sub>2B</sub> increased within 6 h postinjection in RES lungs (2.4-fold increase) and within 2 h postinjection in SUS lungs (3.4-fold increase) (Fig. 2D). Lungs from SUS broilers had higher expression of 5-HT<sub>2B</sub> receptors at 2 h ( $P = 0.016$ ), 6 h ( $P = 0.0088$ ), and 12 h ( $P = 0.0056$ ) postinjection compared with the lungs from RES broilers (Fig. 2D). Serotonin transporter expression increased within 6 h postinjection in RES lungs (2.3-fold increase) or within 2 h postinjection in SUS lungs (2.2-fold increase) and remained elevated at all subsequent time points in both lines. The lines did not differ in serotonin transporter expression at any time points (Fig. 2E).

#### eNOS and iNOS

Expression of eNOS in the lungs of RES broilers increased within 6 h after MP injection (5.4-fold increase), continued to increase up to 12 h postinjection, and remained elevated thereafter. In the lungs of SUS broilers, eNOS expression increased by 2 h postinjection (4.4-fold increase), continued to increase up to 6 h, and remained elevated throughout subsequent time points (Fig. 3A). The lungs of RES broilers had higher eNOS expression at 12 h ( $P = 0.008$ ), 24 h ( $P = 0.0001$ ), and 48 h ( $P < 0.0001$ ) postinjection when compared with the lungs from SUS broilers (Fig. 3A). Expression of iNOS in RES lungs increased by 6 h postinjection (4.7-fold increase), continued to increase up to 24 h, and remained elevated at 48 h, whereas in SUS lungs iNOS expression increased by 6 h, remained at that level at 12 h, and increased again at 24 and 48 h (Fig. 3B). Lungs from RES broilers exhibited higher iNOS expression at 12 h ( $P < 0.0001$ ), 24 h ( $P < 0.0001$ ), and 48 h ( $P < 0.0001$ ) postinjection when compared with the lungs from SUS broilers (Fig. 3B).

#### DISCUSSION

Endothelin-1 elicits vasoconstriction and proliferation of PASMC by binding to ET<sub>A</sub> and ET<sub>B</sub> receptors (19, 40). Plasma levels of endothelin-1 are elevated 3 to 4 times above normal levels in human patients with various forms of PAH including IPAH (39). Lungs of SUS broilers had higher expression of endothelin-1, but the lines did not differ in expression of ET<sub>A</sub>, which indicates that susceptibility to IPAH in broilers is related to higher endothelin-1 expression rather than to ET<sub>A</sub> availability, in agreement with previous studies in which the lungs of broilers with advanced IPAH exhibited higher endothelin-1 expression but lower ET<sub>A</sub> expression than the healthy flock mates (17). The potential impact of ET<sub>B</sub> expression depends on its location. Stimulation of ET<sub>B</sub> receptors on smooth muscle cells results in vasoconstriction (38), and on endothelial cells results in vasodilation (20). In this study, RNA was isolated from whole lung tissue so the ET<sub>B</sub> expression might be from either smooth muscle cells or endothelial cells or both. ET<sub>B</sub> receptors are exclusively involved in the clearance of the circulating endothelin-1 from the blood (10), which reduces the bioavailability of endothelin-1, thereby minimizing its pulmonary vasoconstrictor and mitogenic effects.

Expression of all serotonin receptor types evaluated in this study increased in the lungs of broilers following MP injection. Broilers from the SUS line had a higher expression of 5-HT<sub>1A</sub>

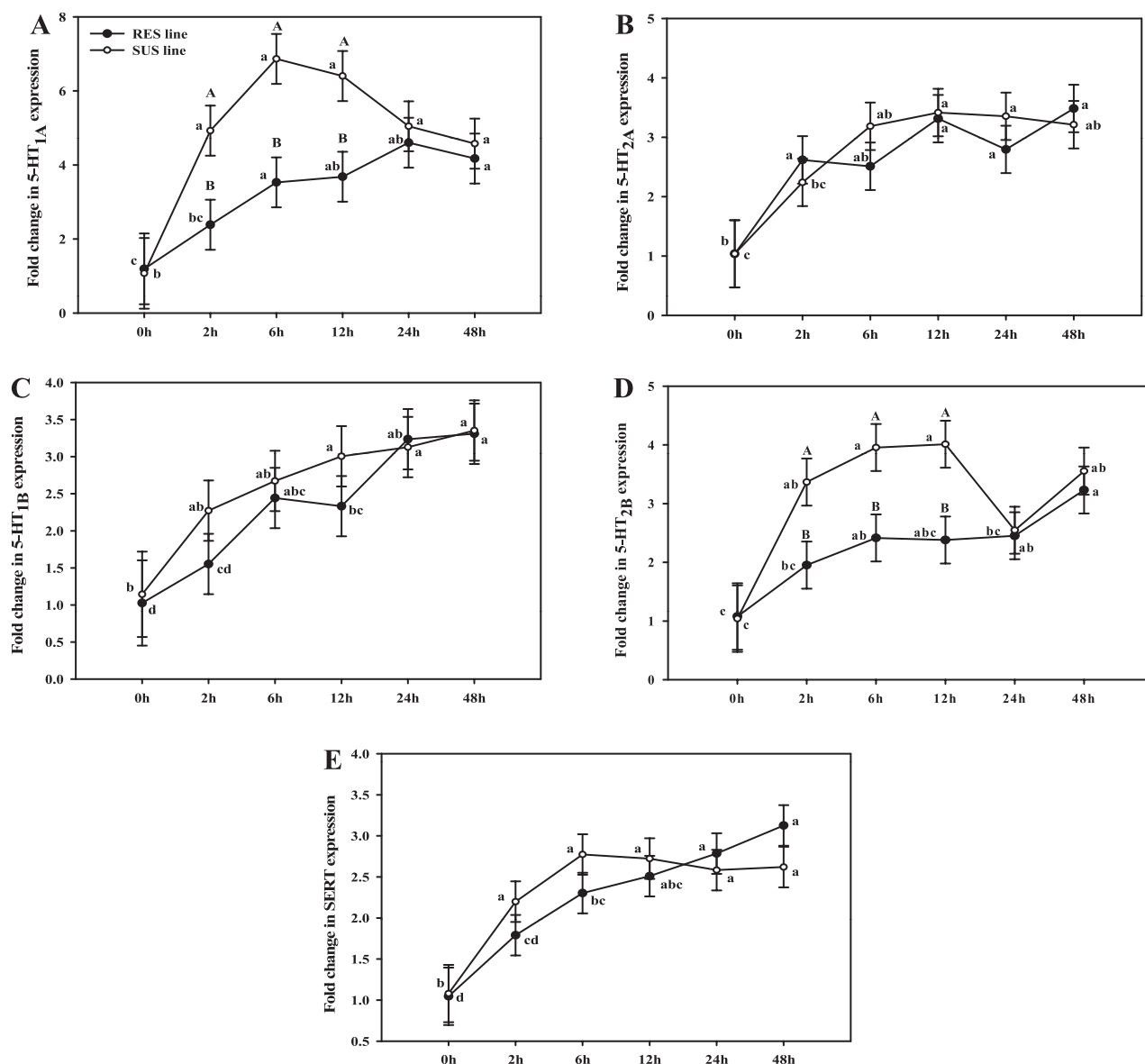


Fig. 2. Fold change in gene expression of serotonin (5-HT) receptors type 1A (5-HT<sub>1A</sub>, A), 2A (5-HT<sub>2A</sub>, B), 1B (5-HT<sub>1B</sub>, C), 2B (5-HT<sub>2B</sub>, D), and serotonin transporter (SERT, E) in lungs of broilers from RES and SUS lines before (0 h) and after (2, 6, 12, 24, and 48 h) intravenous MP injection. Relative gene expression was quantified by real-time RT-PCR using an Applied Biosystems 7300 sequence detection system. The fold change in gene expression was computed by the  $\Delta\Delta C_t$  method, where the MP uninjected cDNA sample was used as the calibrator and 28S cDNA served as an endogenous control. Data are expressed as means  $\pm$  SE ( $n = 6$  per line and time point). Letters A and B indicate line differences between each time point; letters a, b, c, and d indicate differences among the time points within each line. For each analysis, means without a common letter are different ( $P \leq 0.05$ ).

and 5-HT<sub>2B</sub> receptors over the first 12 h postinjection, but the lines did not differ in expression of other receptors. The role of serotonin receptors 5-HT<sub>1B</sub>, 5-HT<sub>2A</sub>, and 5-HT<sub>2B</sub> in vasoconstriction and in proliferation of PASM C has been well established in mammalian IPAH pathogenesis (8, 28). To the best of our knowledge, this is the first evaluation of 5-HT<sub>1A</sub> receptor expression in broiler lungs, and the first indication that 5-HT<sub>1A</sub> receptors may play a role in pathogenesis of IPAH. The higher expression of 5-HT<sub>2B</sub> receptors in the lungs of SUS broilers suggests that this receptor contributes to pulmonary vasoconstriction and PASM C proliferation that accompany susceptibility to IPAH in broilers (5, 61, 62), a phenomenon defined in mammals (34).

The expression of serotonin transporter increased similarly in the lungs of both SUS and RES broilers after the MP injection. Increased serotonin transporter expression enables PASM C to internalize additional serotonin and thereby increases the clearance of serotonin from the circulation and hence reduces serotonin mediated vasoconstriction; but once internalized, serotonin has a mitogenic effect on PASM C leading to vascular remodeling, arterial muscularization, and increase in PVR (11, 26).

Higher expression of eNOS and iNOS should elevate production of nitric oxide, a potent pulmonary vasodilator in mammals and birds (3, 27, see above). Nitric oxide also has an antimitogenic effect on PASM C (44, 45). Blockade of the

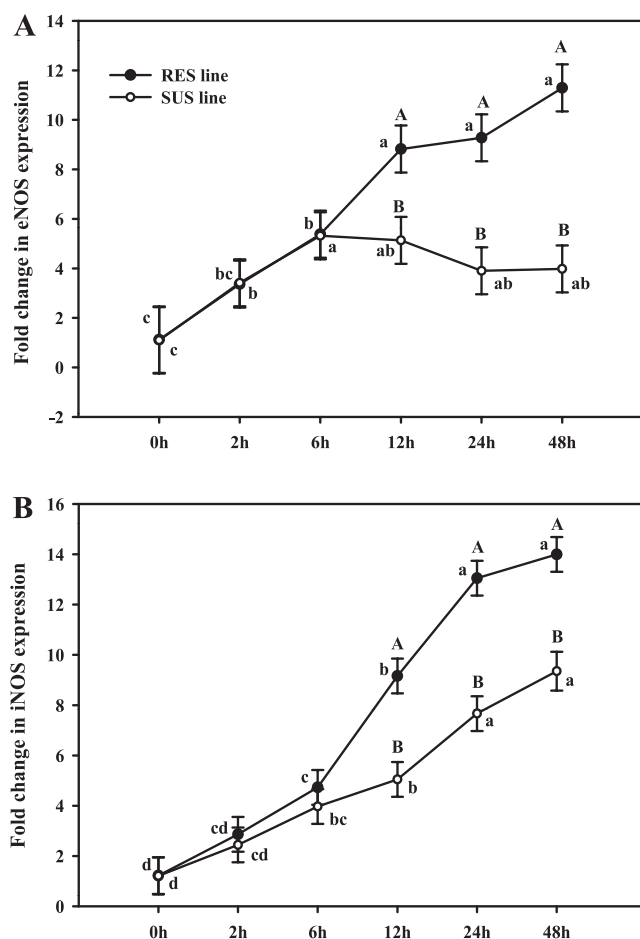


Fig. 3. Fold change in gene expression of endothelial nitric oxide synthase (eNOS, A) and inducible NOS (iNOS, B) in lungs of broilers from the RES and SUS lines before (0 h) and after (2, 6, 12, 24, and 48 h) intravenous MP injection. Relative gene expression was quantified by real-time RT-PCR using an Applied Biosystems 7300 sequence detection system. The fold change in gene expression was computed by the  $\Delta\Delta C_t$  method, where the MP uninjected cDNA sample was used as the calibrator and 28S cDNA served as an endogenous control. Data are expressed as means  $\pm$  SE ( $n = 6$  per line and time point). Letters A and B indicate line differences between each time point; letters a, b, c, and d indicate differences among the time points within each line. For each analysis, means without a common letter are different ( $P \leq 0.05$ ).

activities of eNOS and iNOS by L-NAME decreased the biosynthesis of nitric oxide leading to vasoconstriction and pulmonary hypertension (50, 52, 57, 58). Higher expression of iNOS in the lungs of MP-injected RES broilers compared with SUS broilers supports our previous finding (18). Reduced eNOS expression in the pulmonary arterioles of broilers developing IPAH during chronic exposure to hypobaric hypoxia (30, 31) further underscores the role of eNOS as a modulator of IPAH in broilers. Higher expression of eNOS and iNOS by the lungs of RES broilers provides the distinctive advantage of counteracting multiple pathways through which MP entrapment induces IPAH in SUS broilers. Prostacyclin and prostaglandin  $E_2$  are important pulmonary vasodilators in mammals but are ineffective as vasodilators in broiler chickens (42, 59), leaving nitric oxide as the only known vasodilator, which has a modulatory role in the pathogenesis of IPAH in broilers. The eNOS and iNOS expression patterns following MP injection

support the capacity of nitric oxide to play a key role in the lungs of RES broilers by counteracting vasoconstriction and exerting antimitogenic effect to inhibit PASM proliferation.

In conclusion, in RES broilers the MP elicited higher pulmonary expression of eNOS, iNOS, and  $ET_B$ , which promote vasodilation and inhibit PASM proliferation through the action of nitric oxide. In SUS broilers, the MP elicited higher pulmonary expression of endothelin-1 and the 5-HT $_{2B}$  receptor, which enhance vasoconstriction and PASM proliferation. These results support our hypothesis that the resistance of broiler chickens to IPAH may be due to the higher expression of those vasoactive mediators ( $ET_B$ , eNOS, iNOS) that favor enhanced vasodilation, attenuated vasoconstriction, and reduced PASM proliferation during MP challenges to the pulmonary vasculature.

### Perspectives and Significance

The pathogenesis of PHS in broiler chicken is very similar to human IPAH. Availability of RES and SUS genetic lines of broilers presents an excellent opportunity to study PHS pathogenesis in broiler chickens to further substantiate their use as an animal model for human IPAH. The role of endothelin-1, serotonin, and nitric oxide in PHS in broiler chickens is well known. However, gene expression of these vasoactive mediators in the lung of broilers regarding the pathogenesis of PHS had not been studied. This study demonstrated differential gene expression of key vasoactive mediators in the lungs of clinically healthy broilers from RES and SUS lines following intravenous MP injection. In this study there was no direct evidence that the observed expression of vasoactive mediators translated into protein and functional consequences; nevertheless, these patterns of gene expression are consistent with numerous observations from previous studies. For example, the  $ET_A$  receptor antagonist BQ123 attenuated the development of PH and right ventricular hypertrophy in broilers chronically exposed to low ambient temperature (63). Furthermore, blockade of 5-HT $_{1/2}$  receptors with methiothepin substantially reduced the mortality triggered by injection of MP into SUS broilers (5, 6, 7). Finally, inhibition of eNOS and iNOS with L-NAME increased the mortality triggered by MP injection in broilers (50, 61, 62). This study lays a solid foundation for evaluating the gene and protein expression patterns underlying the development and pathogenesis of PHS in broilers and for validating them as an animal model for IPAH in humans.

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### DISCLOSURE

No conflicts of interest are declared by the author(s).

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# Immune Modulation of the Pulmonary Hypertensive Response to Bacterial Lipopolysaccharide (Endotoxin) in Broilers

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**ABSTRACT** The lungs of broilers are constantly challenged with lipopolysaccharide (LPS, endotoxin) that can activate leukocytes and trigger thromboxane A<sub>2</sub> (TxA<sub>2</sub>)- and serotonin (5HT)-mediated pulmonary vasoconstriction leading to pulmonary hypertension. Among broilers from a single genetic line, some individuals respond to LPS with large increases in pulmonary arterial pressure, whereas others fail to exhibit any response to the same supramaximal dose of LPS. This extreme variability in the pulmonary hypertensive response to LPS appears to reflect variability in the types or proportions of chemical mediators released by leukocytes. Our research has confirmed that TxA<sub>2</sub> and 5HT are potent pulmonary vasoconstrictors in broilers and that broilers hatched and reared together consistently exhibit pulmonary hypertension after i.v. injections of TxA<sub>2</sub> or 5HT. Previous in vitro studies conducted using macrophages from different lines of chickens demonstrated innate variability in the LPS-stimulated induction of nitric oxide synthase (iNOS)

followed by the onset of an LPS-refractory state. The NOS enzyme converts arginine to citrulline and nitric oxide (NO). It is known that NO produced by endothelial NOS serves as a key modulator of flow-dependent pulmonary vasodilation, and it is likely that NO generated by iNOS also contributes to the pulmonary vasodilator response. Accordingly, it is our hypothesis that the pulmonary hypertensive response to LPS in broilers is minimal when more vasodilators (NO, prostacyclin) than vasoconstrictors (TxA<sub>2</sub>, 5HT) are generated during an LPS challenge. Indeed, inhibiting NO production through pharmacological blockade of NOS with the inhibitor N<sup>ω</sup>-nitro-L-arginine methyl ester modestly increased the baseline pulmonary arterial pressure and dramatically increased the pulmonary hypertensive response to LPS in all broilers evaluated. Innate differences in the effect of LPS on the pulmonary vasculature may contribute to differences in susceptibility of broilers to pulmonary hypertension syndrome (ascites).

(*Key words:* ascites, prostacyclin, macrophage, nitric oxide, thromboxane)

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## PULMONARY VASCULAR CAPACITY AND PULMONARY HYPERTENSION

The pulmonary vascular capacity of modern broiler chickens is only marginally adequate to accommodate the cardiac output (CO) required to support the metabolic demands incurred by fast growth and the extremes of environmental temperatures (Julian, 1989; Peacock et al., 1989; Wideman and Bottje, 1993; Owen et al., 1995; Wideman et al., 1998c; 2003a,b; Wideman, 2000, 2001; Wideman and Tackett, 2000). In this context, the “pulmonary vascular capacity” can be broadly defined to encompass metabolic limitations related to the tone (degree of contraction) maintained by the primary resistance vessels (precapillary arterioles) within a broiler’s lungs, as well as anatomical constraints related to the compliance and effective

volume of the blood vessels. Hemodynamic evaluations of broilers have confirmed that their pulmonary blood vessels have a low compliance (are poorly distensible), the vascular channels function as if they are fully engorged with blood (additional vascular volume cannot be recruited), and the precapillary arterioles are the primary sites of excessive resistance to pulmonary blood flow (Wideman and Kirby, 1995a,b; Wideman et al., 1996a,b, 1999b; Forman and Wideman, 1999, 2001; Chapman and Wideman, 2001). Accordingly, broilers are susceptible to the onset of pulmonary hypertension leading to pulmonary hypertension syndrome (PHS, ascites) whenever

**Abbreviation Key:** CO = cardiac output; ET-1 = endothelin 1; eNOS = endothelial nitric oxide synthase; IL = interleukin; iNOS = inducible nitric oxide synthase; L-NAME = N<sup>ω</sup>-nitro-L-arginine methyl ester; LPS = lipopolysaccharide; mCD14 = membrane bound CD14 receptor; NO = nitric oxide; NOS = nitric oxide synthase; PAF = platelet activating factor; PAP = pulmonary arterial pressure; PGI<sub>2</sub> = prostacyclin; PHS = pulmonary hypertension syndrome; PIM = pulmonary intravascular macrophages; PVR = pulmonary vascular resistance; TLR4 = toll-like receptor 4; TxA<sub>2</sub> = thromboxane A<sub>2</sub>; 5HT = 5-hydroxytryptamine (serotonin).

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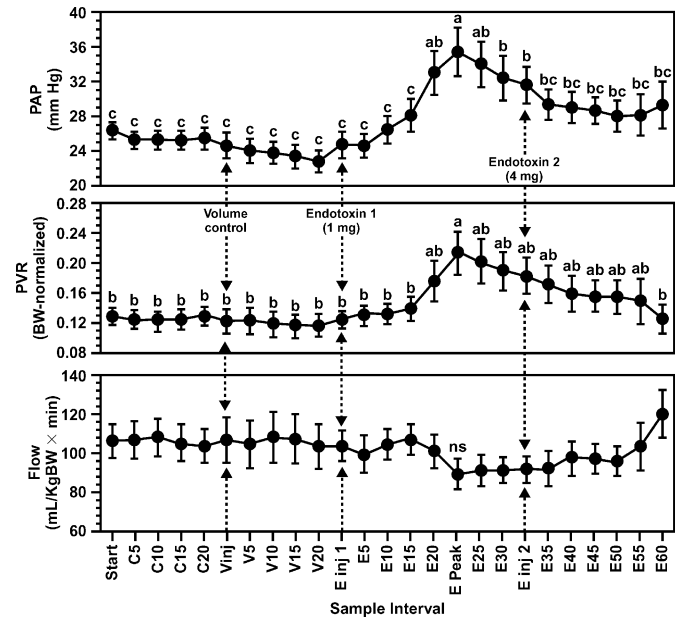
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their right ventricle must develop an elevated pulmonary arterial pressure (PAP) to propel the CO through lungs having a inadequate pulmonary vascular capacity and thus an elevated pulmonary vascular resistance (PVR) (Wideman and Bottje, 1993; Wideman, 2000, 2001). Indeed, experimental procedures designed specifically to reduce the pulmonary vascular capacity and elevate the PVR have been shown to reliably initiate the characteristic pathogenesis leading to PHS in susceptible broilers (Wideman and Kirby, 1995a, 1996; Wideman et al., 1997, 2002; Ruiz-Feria et al., 1999; Wideman and Erf, 2002). In contrast, some broilers are genetically resistant to PHS and are capable of thriving in spite of substantial reductions in their pulmonary vascular capacity (Wideman and French, 1999; Wideman et al., 2002). Resistant male and female breeder parents selected for their robust pulmonary vascular capacity produced progeny exhibiting a cumulative 90% reduction in susceptibility to PHS when grown as rapidly as possible during exposure to cool temperatures (Wideman and French, 2000). The rapid progress achieved in selecting PHS-resistant broiler lines demonstrates that selection pressure rigorously focused to chronically challenge the pulmonary vasculature succeeded in virtually eliminating a gene coding for a highly significant proportion of the PHS susceptibility in commercial broilers (Wideman, 2001).

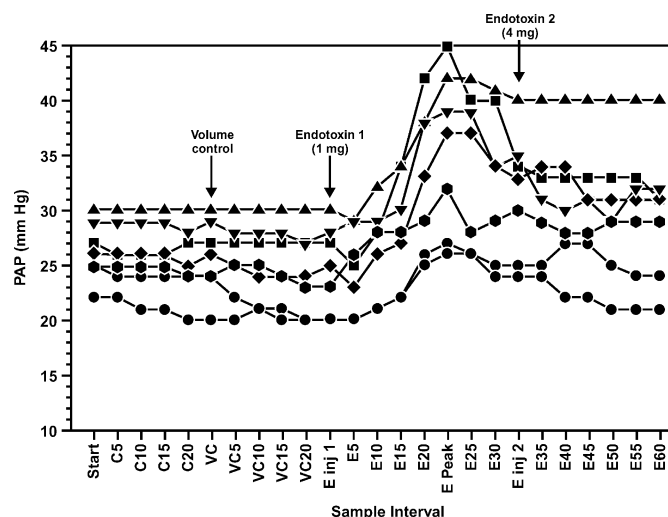
## LIPOPOLYSACCHARIDE (ENDOTOXIN) AND PULMONARY HYPERTENSION

All factors contributing to increases in the PVR theoretically can initiate or accelerate the pathogenesis of PHS if the right ventricle of the heart is forced to increase the PAP to propel the requisite CO through the lungs (Wideman and Bottje, 1993; Wideman, 2000). Bacterial lipopolysaccharide (LPS, endotoxin) is an integral component of the cell wall of gram-negative bacteria, including *Escherichia coli* and a number of other pathogens (e.g., *Salmonella*, *Bordetella*, *Campylobacter*). In mammals, LPS stimulates pulmonary vasoconstriction and pulmonary hypertension (accompanied by increases in PVR and PAP, respectively) mediated by endothelin-1 (ET-1) and thromboxane A<sub>2</sub> (TxA<sub>2</sub>) (Morel et al., 1989; Longworth et al., 1994; Staub, 1994; Faltin et al., 1996; Snapper et al., 1998; Wanecek et al., 2000). Our research has confirmed that LPS can cause pulmonary vasoconstriction and pulmonary hypertension in broilers. As shown in Figure 1, clinically healthy broilers injected i.v. with a supramaximal (1 mg) dose of LPS exhibited a delayed-onset ( $\geq 15$  min) pulmonary vasoconstriction that increased the pulmonary arterial pressure within 20 to 25 min followed by a gradual decline by 60 min postinjection. Subsequent to the onset of pulmonary hypertension, the same birds became refractory or tolerant (unresponsive) to injections of  $\geq 4$  mg LPS (Wideman et al., 2001). The time-course and magnitude of the pulmonary hypertensive responses of individual broilers to LPS varied widely for reasons that were not apparent based on attempts to maintain uniformity across all aspects of the experiment. Among broilers



**FIGURE 1.** Pulmonary arterial pressure (PAP, upper panel), pulmonary vascular resistance (PVR, middle panel), and blood flow through the right pulmonary artery (Flow, lower panel) for male broilers (mean  $\pm$  SEM,  $n = 7$ ) at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (Vinj), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the injection with 1 mg of endotoxin (E inj 1), at 5-min intervals after the injection with 1 mg of endotoxin (E5 to E30), during the maximum PAP response to 1 mg of endotoxin (E Peak), within 30 s after the injection with 4 mg of endotoxin (E inj 2), and at 5-min intervals thereafter (E35 to E60). <sup>a-c</sup>Different letters designate differences between means over time ( $P \leq 0.05$ ); ns = not significant ( $P > 0.05$ ) (adapted from Wideman et al., 2001).

from a single genetic line that had been hatched and reared together, some hyperresponsive individuals reacted to LPS with large increases in PAP, whereas the same supramaximal dose of LPS failed to elicit pulmonary hypertension in nonresponsive individuals (Figure 2) (Wideman et al., 2001). Subsequent research confirmed that broilers are equally variable in their responses to similar doses of LPS purified from *E. coli* and *S. typhimurium*, and doses of LPS ranging from 20  $\mu$ g/kg BW to 10 mg/kg BW were capable of triggering maximal pulmonary hypertension in hyper- but not hyporesponsive individuals (R. F. Wideman, M. E. Chapman, W. Wang, and G. F. Erf, unpublished observations). Attempts to reduce the individual variability in the pulmonary hypertensive response to LPS were not effective. Broilers reared in clean stainless steel cages from which the fecal and dander material were removed daily nevertheless exhibited pulmonary hypertensive responses to LPS that were as variable as those observed in broilers whose pulmonary gas exchange capacity had been compromised by their being reared at a higher density on floor litter (Wang et al., 2002b). Rearing broilers on litter increases the oxidative stress, structural damage to the lungs, and the incidence of birds found to have viable intrapulmonary microorganisms when compared with broilers reared in cages or on raised netting floors (Madelin and Wathes, 1989; Bottje et al., 1998). Broilers whose lungs were primed 48 h pre-



**FIGURE 2.** Individual pulmonary arterial pressure (PAP) values for 7 male broilers at the start of data collection (Start), at 5-min intervals during the control period (C5 to C20), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 to VC20), within 30 s after the injection with 1 mg of endotoxin (E inj 1), at 5-min intervals after the injection with 1 mg of endotoxin (E5 to E30), during the maximum PAP response to 1 mg of endotoxin (E Pk), within 30 s after the injection with 4 mg of endotoxin (E inj 2), and at 5-min intervals thereafter (E35 to E60) (adapted from Wideman et al., 2001; also see Wang et al., 2002a,b).

viously by intravenously injecting a low dose (minimally occlusive) of cellulose microparticles did not differ in the time of onset, amplitude, or variability in their pulmonary hypertensive response to LPS when compared with unprimed controls (Wang et al., 2002a). Intravenously injected cellulose microparticles become lodged in the pulmonary precapillary arterioles and initiate acute focal inflammatory responses within the surrounding lung parenchyma (Wideman et al., 2002; Wang et al., 2003). These observations suggest the variation in pulmonary vascular responsiveness to LPS among individuals within a broiler population may reflect the innate rather than acquired characteristics of those individuals.

## IMMUNE MODULATION OF LPS-INDUCED PULMONARY HYPERTENSION

The lungs of chickens are constantly challenged with airborne gram-negative bacteria and LPS, as well as LPS translocated from pathogens resident in the intestine (Whyte, 1993, 2002; Sander, 1994; Martensson, 1995; Brown et al., 1997; Alexander and Rietschel, 2001). Approximately 40% of the gram-negative bacteria in poultry house dust reside on particles of a respirable size ( $<5 \mu\text{m}$  in diameter) capable of reaching the air sacs and gas exchange regions (parabronchi) of avian lungs. Substantial quantities of aerosolized LPS (e.g.,  $0.31 \mu\text{g}/\text{m}^3$  of air) are consistently detected in the air of poultry houses (Hayter and Besch, 1974; Sander, 1994). Our current understanding of the avian intrapulmonary immune responses to aerosolized gram-negative bacteria and LPS is limited. Most aerosolized particulates are trapped by

mucus in the conducting airway system which includes the trachea and primary and secondary bronchi. These particulates are prevented from entering the lung parenchyma by the mucociliary "escalator" present in the conducting airways and at the lung-air sac junction of chickens. The mucus and entrapped particulates are propelled by cilia to the pharynx for elimination or ingestion (McLelland, 1989a,b; Jeurissen et al., 1994; Fedde, 1998). The lymphoid tissues of bronchi primarily consist of demarcated oval lymphoid nodules present directly underneath the bronchiolar epithelial layer (Sminia et al., 1989; Jeurissen et al., 1994). This bronchus-associated lymphoid tissue can be most frequently observed at the junction of primary and secondary bronchi. The tissue has defined T and B cell areas, germinal centers, and accessory cells required for antigen presentation, suggesting a role of this tissue in the initiation of mucosal immune responses. Exposure to ammonia inhibits mucociliary transport, contributes to deciliation of the conducting airways, promotes leukocyte infiltration into the lung parenchyma, and increases the susceptibility of chickens to airborne pathogens (Anderson et al., 1964, 1966; Al-Mashhadani and Beck, 1985). In broilers treated with infectious bronchitis virus to inhibit mucociliary transport and permit the bacteria to penetrate through the conducting airways to the lung parenchyma, intratracheal inoculation with *E. coli* increased the incidence of PHS 5-fold (Tottori et al., 1997; Yamaguchi et al., 2000).

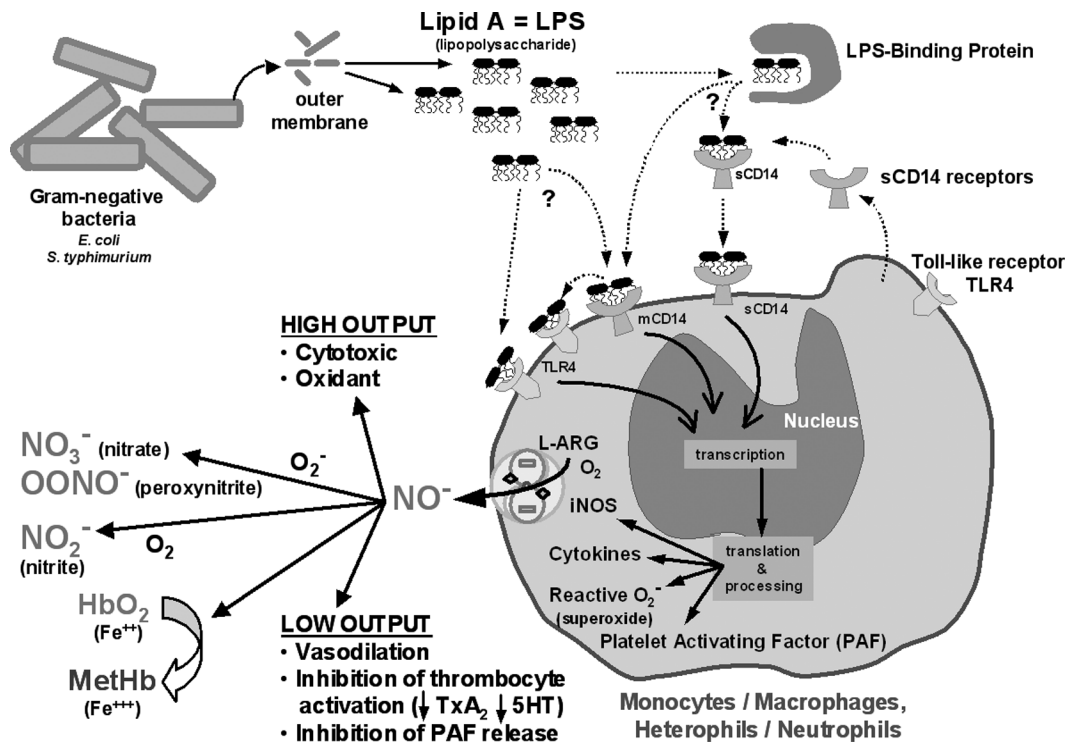
Macrophages and neutrophils play a central role in the mammalian response to LPS, and mammals have alveolar macrophages as a first line of defense at their gas exchange surfaces. Birds do not appear to have resident macrophages or other resident leukocytes at their gas exchange surfaces or within the air sacs. However, during respiratory infection or aspiration of particulates, leukocytes (primarily phagocytic macrophages and heterophils) are present in lavage fluid from the avian respiratory tract, indicating mechanisms do exist that allow these cells to enter the gas-filled spaces when necessary (e.g., inflammation) (Ficken et al., 1986; Toth and Siegel, 1986; Toth et al., 1987, 1988; Qureshi et al., 1993; Klika et al., 1996; Kunkle and Rimler, 1996; Pruimboom et al., 1996; Brown et al., 1997). Lung tissues surrounding the noncartilaginous, nonmucociliary tertiary bronchi (parabronchi) contain dendritic cells that may be important in the uptake and presentation of airborne antigens penetrating the parabronchus (Jeurissen et al., 1994). Immune cells are also present in the parenchyma of the lung. These include T and B cells as well as dendritic cells, macrophages, and mast cells (Jeurissen et al., 1994; Wang et al., 2003). In view of the normal dearth of macrophages on the gas surfaces of the air sac and parabronchial epithelium, it has been proposed that in birds the initial phagocytic function may reside in the pulmonary epithelial cells themselves followed by exocytosis to the underlying interstitium (Brown et al., 1997). For example, respirable microparticulates reaching the lower respiratory tract of birds were trapped in the surfactant (trilamellar) layer, engulfed by the gas-exchange epithelial cells, and translo-

cated to the interstitium where they were phagocytosed by macrophages (Bretz and Schmidt-Nielsen, 1971; Bland et al., 1985; Stearns et al., 1987; Brown et al., 1997). A role of the surfactant and epithelium of mammalian lungs in the local defense against LPS is also supported by recent evidence (Sano et al., 1999; Dentener et al., 2000; Song and Phelps, 2000).

The lungs also respond immunologically to bloodborne particulates and antigens. An important yet under-appreciated function of the pulmonary vasculature is to filter and clear the returning venous blood of micro- and macroparticulate matter including bacteria, immune complexes, cellular debris, aged red blood cells, and emboli. In addition to particulates entering the blood stream directly, materials engulfed by lymphatic capillaries throughout the body subsequently flow through major lymph trunks to empty into the vena cava immediately proximal to the right atrium (Berens and Rautenfeld, 1993). The pulmonary vasculature of broilers therefore can be challenged by a wide variety of substances, and the ability of the pulmonary vasculature to clear these substances from the blood serves to defend sensitive tissues such as the brain and heart. In several mammalian species, bloodborne antigens and intravenously injected microparticulates circulating through the pulmonary capillaries are primarily removed from the blood stream by pulmonary intravascular macrophages (PIM) which are large mature macrophages bound to the pulmonary capillary endothelium. The PIM of sheep are responsible for 40 to 100% of uptake of intravenously injected particulates and have been shown to occupy 15% of the intravascular volume of pulmonary capillaries. Collectively PIM provide an extensive surface area for contact with bloodborne antigens and constitute an important part of the mononuclear phagocytic system (Warner et al., 1986). However, resident PIM capable of removing tracer particulates and bacteria evidently are not present in rats and chickens (Lund et al., 1921; Malik, 1983; Warner et al., 1986; Winkler, 1988; Staub, 1994; Dantzer, 1997; Heffner and Repine, 1997; Brain et al., 1999; Weidner and Lancaster, 1999). The absence of PIM does not leave the chicken's lungs immunologically unresponsive to bloodborne antigens because CO values for broilers demonstrate that the entire blood volume, and thus all of the circulating leukocytes, flow through the lungs every 30 s (Sturkie, 1986; Wideman, 1999). Indeed, intravenously injected microparticulates and LPS induce dynamic intrapulmonary inflammatory responses in broilers. Preliminary histopathology of lung tissues obtained after LPS injections revealed vascular congestion, endothelial cell swelling, and notable increases in both large and small mononuclear cells within in the pulmonary microvasculature. Intravenous injections of microparticulates and LPS cause acute reductions in circulating monocyte concentrations that coincide with the appearance of monocytes/macrophages in the lung parenchyma of broilers (Wideman et al., 2002; Wang, 2003; Wang et al., 2003). Intravenous LPS administration greatly enhances the pulmonary mononuclear cell uptake of circulating particles and pathogens

in the rat (Warner et al., 1994), and pretreatment of chickens with LPS apparently activated circulating leukocytes and enabled the microparticulate tracer Monastral blue to trigger a profound pulmonary hypertension that could not be elicited with injections of the tracer alone (Weidner and Lancaster, 1999). Similarly, LPS injected into chicken skin resulted in uptake of carbon particles by mononuclear cells in venules near the injection site, a process not observed in skin injected with saline alone. Other effects of LPS injury in chicken skin included increased vascular permeability for up to 30 min postinjection and leukocyte emigration (Katiyar et al., 1992). Avian thrombocytes are phagocytic toward bacteria and microparticulates (Glick et al., 1964; Carlson et al., 1968; Sterz and Weiss, 1973; Chang and Hamilton, 1979a,b; Awadhiya et al., 1980; Ohata and Ito, 1986; DaMatta et al., 1998; Roland and Birrenkott, 1998), and in mammals LPS causes an acute intrapulmonary platelet aggregation accompanied by the release of 5-hydroxytryptamine (5HT, serotonin), a potent pulmonary vasoconstrictor (Shibazaki et al., 1996, 1999). The pulmonary hypertensive response to LPS in broilers may implicate intrapulmonary thrombocyte accumulation and 5HT release (Wang, 2003). Hence it is likely that in chickens, as in the rat, LPS causes the release of mediators by pulmonary endothelial cells and circulating leukocytes leading to an influx of other inflammatory cells into the lung parenchyma.

The LPS-initiated intrapulmonary inflammatory responses having a major impact on the pulmonary vasculature are summarized in Figures 3 to 5. The biological activity characteristic of endotoxin resides in the Lipid A component of the outer membrane of gram-negative bacteria. LPS entering the blood first binds to LPS binding protein in the plasma which in turn transfers the LPS to evolutionarily ancient CD14 "pattern recognition receptors" that are part of the innate (genetically predetermined, non-clonal) immune system. The binding protein transfers LPS to membrane-bound CD14 receptors (mCD14) on the surface of monocytes/macrophages and neutrophils/heterophils. Binding of LPS to mCD14 receptors facilitates the recognition of LPS by membrane-associated toll-like receptors (TLR4), and mCD14 receptors may transfer LPS to TLR4 receptors. LPS also can bind to soluble CD14 receptors that are synthesized by monocytes/macrophages and then released into the plasma (Figure 3). The LPS-soluble CD14 receptor complex appears to be capable of directly activating local endothelial cells and platelets/thrombocytes (Figures 3 and 4). The ensuing cascade of intracellular signaling events culminates in transcription and translation of genes associated with the innate immune responses; production and local release of inflammatory cytokines (e.g., interleukin(IL)-1, IL-6, and tumor necrosis factor- $\alpha$ , ET-1, and platelet activating factor (PAF); release of 5HT from platelets; and synthesis of a wide range of eicosanoid metabolites consisting of leukotrienes, prostaglandins (prostaglandin, PGI<sub>2</sub>), and TxA<sub>2</sub> (Figures 3 and 4) (Shibazaki et al., 1996, 1999; Chilton et al., 1997; Arditi, 1999; Kuijpers and Van

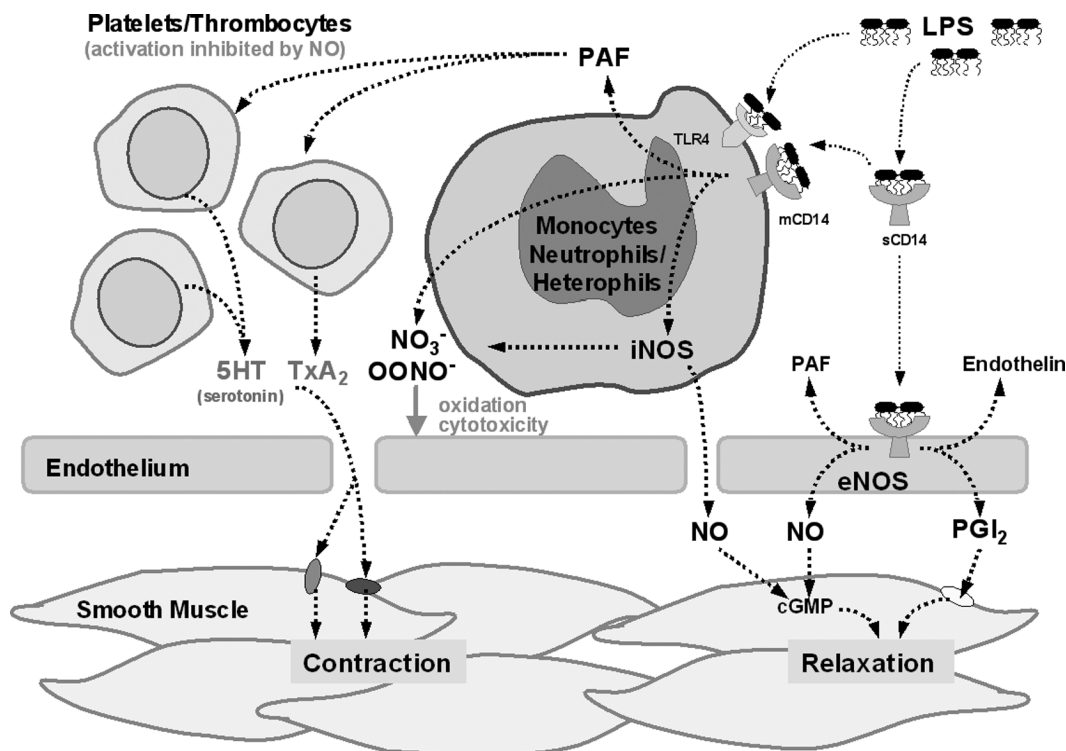


**FIGURE 3.** Characteristic endotoxin activity resides in the lipid A component (lipopolysaccharide, LPS) of the outer membrane of gram-negative bacteria. LPS binds to LPS binding protein which in turn transfers the LPS to CD14 "pattern recognition receptors" that are membrane-bound (mCD14) on the surface of monocytes/macrophages and neutrophils/heterophils or that are synthesized and released by monocytes/macrophages into the plasma as soluble CD14 receptors (sCD14). Binding of LPS to CD14 receptors facilitates the recognition of LPS by membrane-associated toll-like receptors (TLR4). The ensuing cascade of intracellular signaling events culminates in transcription and translation of genes associated with the innate immune responses, production and local release of inflammatory cytokines and platelet activating factor (PAF), and induction of the gene for nitric oxide synthase (iNOS). Once activated, iNOS produces copious quantities of nitric oxide (NO) from L-arginine. NO and its derivative reactive oxygen/nitrogen species (e.g., nitrate, peroxynitrite, nitrite) are nonspecifically cytotoxic to pathogens but also can damage tissues in the immediate vicinity of the inflammatory response. NO and its metabolites also combine with hemoglobin to form nitrosyl-hemoglobin complexes that are oxidized to methemoglobin and therefore reduce the oxygen carrying capacity of blood. NO also promotes flow-dependent pulmonary vasodilation, inhibits activation of thrombocytes by platelet activating factor (PAF), and inhibits thrombocyte release of thromboxane (TXA<sub>2</sub>) and serotonin (5-hydroxytryptamine, 5HT) (figure adapted from Raetz and Whitfield, 2002).

der Poll, 1999; Tobias, 1999; Alexander and Rietschel, 2001; Gryglewski et al., 2001).

Lipopolysaccharide also induces leukocytes to express the gene for nitric oxide synthase (NOS) (Figure 3) (Chang et al., 1996; Hussain and Qureshi, 1997, 1998; Dil and Qureshi, 2002a,b; Janeway and Medzhitov, 2002; Qureshi, 2003). Previously NOS had been considered a "constitutive" (constantly expressed) enzyme in endothelial cells (NOS-3, eNOS) and an "inducible" (acutely upregulated) enzyme in monocytes/macrophages and neutrophils/heterophils (NOS-2, iNOS). Current evidence indicates that NOS can be induced in a variety of cells including endothelial cells. The eNOS enzyme tends to be activated transiently due to its readily reversible binding of the Ca<sup>2+</sup>/calmodulin complex, whereas the iNOS enzyme binds its Ca<sup>2+</sup>/calmodulin complex so tightly that, once activated, iNOS produces copious quantities of nitric oxide (NO) (Davis and Matalon, 2001). The NOS enzyme forms the free radical NO from the guanidine nitrogen of L-arginine, an essential amino acid for birds and a limiting substrate for avian NOS and macrophage function (Taylor et al., 1992; Dietert et al., 1994; Wideman et al., 1995; Martinez-Lemus et al., 1999; Kidd et al., 2001; Ruiz-Feria et al., 2001; Villamor et al., 2002). Evidence

that supplemental L-arginine can reduce PAP and PVR during endotoxemia in mammals may indicate that LPS can stimulate sustained rates of NO production that are high enough to deplete extracellular reserves of the L-arginine substrate (Weitzberg, 1993). NO and its derivative reactive oxygen/nitrogen species (e.g., nitrate, peroxynitrite, nitrite) are nonspecifically cytotoxic to pathogens, but also can damage tissues in the immediate vicinity of the inflammatory response, thereby contributing to the pathogenesis of PHS in broilers (Bottje and Wideman, 1995; Gaston and Stamler, 1997). NO and its metabolites also combine with hemoglobin to form nitrosyl-hemoglobin complexes that are oxidized to methemoglobin and therefore reduce the oxygen carrying capacity of blood (Gaston and Stamler, 1997). Hemoglobin is capable of binding NO in quantities sufficient to inhibit NO-mediated influences on the systemic arterial vasculature of chickens (Hasegawa et al., 1993) (Figure 3). As shown in Figure 5, endothelial-derived NO diffuses into the vascular smooth muscle where it activates soluble guanylate cyclase to increase intracellular concentrations of guanosine 3',5'-cyclic monophosphate and promote flow-dependent pulmonary vasodilation (relaxation of vascular smooth muscle). NO relaxes pre-constricted pul-

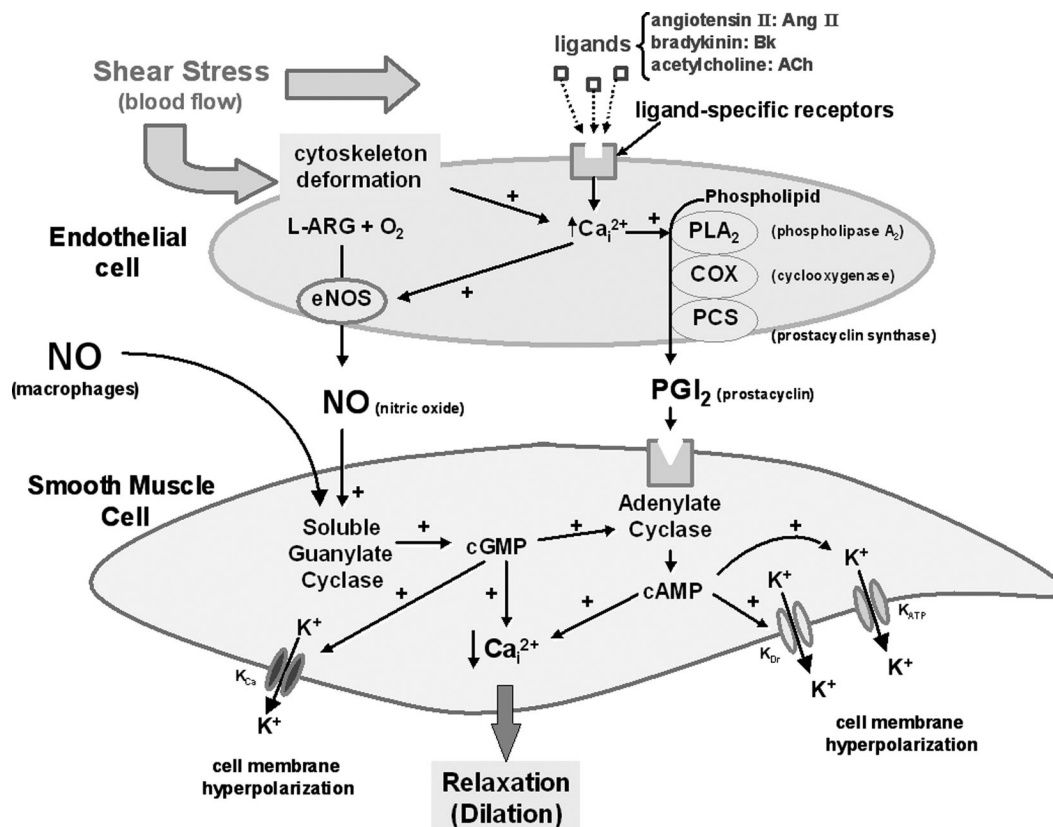


**FIGURE 4.** Lipopolysaccharide (LPS) binds to membrane-bound receptors (mCD14) on the surface of monocytes/macrophages and neutrophils/heterophils or to soluble receptors (sCD14) that can activate the endothelium. The CD14 receptors and toll-like receptors (TLR4) trigger a cascade of intracellular signaling events including the release of platelet activating factor (PAF), endothelin, and induction of nitric oxide synthase (iNOS). PAF causes thrombocytes to release substances that constrict pulmonary vascular smooth muscle (e.g., thromboxane:  $\text{TxA}_2$ , and serotonin: 5HT). The iNOS enzyme produces copious quantities of nitric oxide (NO) and derivative reactive oxygen/nitrogen species (e.g.,  $\text{NO}_3^-$ ,  $\text{OONO}^-$ ) that are nonspecifically cytotoxic. NO and prostacyclin ( $\text{PGI}_2$ ) relax pulmonary vascular smooth muscle, NO inhibits PAF activation of thrombocytes, and NO and  $\text{PGI}_2$  inhibit platelet aggregation and the formation of obstructive microthrombi.

monary artery rings isolated from broiler and Leghorn chickens, and NO minimizes the onset of pulmonary hypertension when broiler lungs are challenged in vivo with disproportionate increases in CO (Wideman et al., 1995, 1996a, 1998b; Martinez-Lemus et al., 1999; Villamor et al., 2002). Therefore it is reasonable to assume that NO generated by leukocytes in response to LPS can dilate the smooth muscle of the pulmonary vasculature and airways, thereby helping to attenuate the pulmonary hypertensive response to LPS (Figure 5).

Processes that initiate intrapulmonary inflammatory responses are recognized as potentially being profoundly deleterious to pulmonary hemodynamics and gas exchange in mammals (Malik, 1983; Dantzker, 1997; Heffner and Repine, 1997). Once an intrapulmonary inflammatory response has been initiated, the extent of the subsequent lung injury is determined by the proportions of "damaging" vs. "protective" factors released by leukocytes and local tissues. Factors that promote lung damage and cause excessive constriction of pulmonary blood vessels and airways include PAF, pro-inflammatory cytokines (e.g., IL-6), reactive oxygen species, ET-1,  $\text{TxA}_2$ , and 5HT. The leukocytes and endothelial cells also produce vasodilators,  $\text{PGI}_2$  and NO, that are considered "protective" of pulmonary function to the extent that they promote smooth muscle relaxation and attenuate pulmonary hypertension. For example, when mammals are infused i.v.

with LPS, both the vasoconstrictor  $\text{TxA}_2$  and the vasodilator  $\text{PGI}_2$  normally are produced and can exert dynamically antagonistic influences on pulmonary vascular resistance.  $\text{PGI}_2$  and NO inhibit both platelet aggregation and the formation of vascular microthrombi that otherwise can physically occlude the vascular bed and progress to the disseminated intravascular coagulation, ischemia, and tissue destruction observed in sepsis (Radomski et al., 1987; Wanecek et al., 2000). NO also reduces or inhibits the concurrent local LPS-stimulated release of PAF, ET-1,  $\text{TxA}_2$ , and 5HT, thereby minimizing the tissue damage and vasoconstriction attributable to leukocyte activation (Figures 3 and 4) (Longworth et al., 1994; Frank et al., 1996; Gaston and Stamler, 1997; Teder and Nobel, 2000; Davis and Matalon, 2001; Gryglewski et al., 2001; Miyata et al., 2001; Lauer et al., 2002). For example, pharmacological blockade of NOS with the inhibitor  $\text{N}^w$ -nitro-L-arginine methyl ester (L-NAME) permitted LPS to stimulate unrestricted platelet and neutrophil activation leading to vascular and airway constriction, acute microvascular lung injury, and hemorrhagic lung edema (Gryglewski et al., 2001). Naturally occurring NO donors, such as S-nitrosoglutathione, reduce the pulmonary vasoconstrictive response to 5HT through possible chemical modification by NO of the 5HT<sub>2</sub> G protein-coupled receptor system (Nozik-Grayck et al., 2002). NO currently is recognized as a key protective modulator of leukocyte-mediated

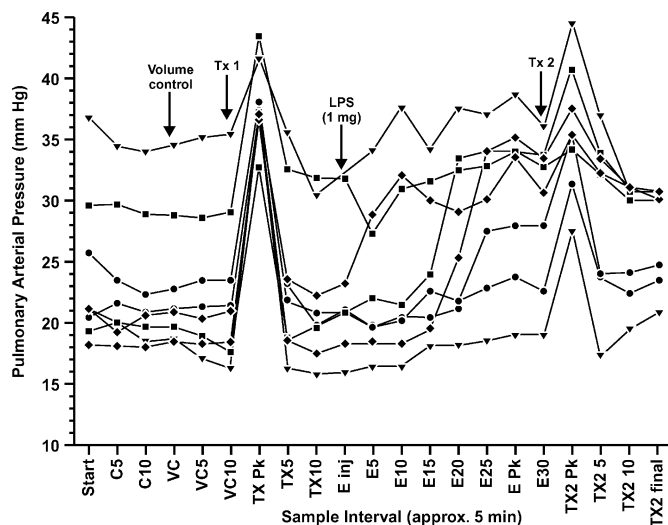


**FIGURE 5.** Elevated blood flow rates apply shear stress that deforms the cytoskeleton of endothelial cells causing intracellular free calcium ion ( $\text{Ca}_i^{2+}$ ) concentrations to increase. The  $\text{Ca}_i^{2+}$  concentrations also increase in response to binding of endothelium-dependent pulmonary vasodilators (e.g., angiotensin II, Ang II; bradykinin, Bk; and acetylcholine, ACh) to their specific membrane receptors. The  $\text{Ca}_i^{2+}$  activates endothelial nitric oxide synthase (eNOS) and phospholipase A<sub>2</sub> (PLA<sub>2</sub>) leading to increased synthesis of nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>), respectively. NO derived from the endothelium or from leukocytes (e.g., macrophages) readily diffuses into vascular smooth muscle cells where it activates soluble guanylate cyclase to increase intracellular concentrations of guanosine 3',5'-cyclic monophosphate (cGMP). PGI<sub>2</sub> binds to its receptor on the smooth muscle cell membrane and activates adenylate cyclase leading to the production of adenosine 3',5'-cyclic monophosphate (cAMP). Both cGMP and cAMP promote  $\text{Ca}_i^{2+}$  and  $\text{K}^+$  efflux from smooth muscle cells, thereby preventing calmodulin-mediated actin-myosin interactions (reduced  $\text{Ca}_i^{2+}$ ) and hyperpolarizing the cell ( $\text{K}^+$  efflux). The result is pulmonary vasodilation (relaxation of vascular smooth muscle throughout the lungs).  $\text{K}_{\text{Ca}}$  =  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channel;  $\text{K}_{\text{ATP}}$  = ATP-dependent  $\text{K}^+$  channel;  $\text{K}_{\text{Dr}}$  = delayed rectifier  $\text{K}^+$  channel (figure adapted from Hecker, 2000; also see Villamor et al., 2002).

damage and thrombosis within the pulmonary circulation of mammals, and it appears likely that NO rather than PGI<sub>2</sub> serves as the primary intrapulmonary modulator of the detrimental effects of PAF and TxA<sub>2</sub> (Grabarevic et al., 1997). NO also may play a protective role in chickens. The addition of L-NAME causes chicken pulmonary artery rings to contract, thereby revealing a sustained role of NO in reducing the basal tone of the vascular smooth muscle. Endothelium-dependent relaxation of chick pulmonary artery rings primarily depends on NO rather than PGI<sub>2</sub> production (Villamor et al., 2002). Supplemental L-arginine and NO attenuated the response of chicken pulmonary artery rings to receptor-mediated endothelium-dependent vasoconstrictors such as ET-1 and the TxA<sub>2</sub> mimetic U-46619 (Martinez-Lemus et al., 1999; Villamor et al., 2002). Injecting L-NAME i.v. into intact broilers caused a modest pulmonary hypertension that was counteracted by administering the NOS-independent NO donor sodium nitroprusside (Weidong et al., 2002), and repeated i.p. injections of L-NAME caused PHS in broilers (Grabarevic et al., 1997).

## CURRENT KNOWLEDGE, HYPOTHESES, AND ONGOING RESEARCH

The evidence currently available supports the hypothesis that variability in the pulmonary hypertensive responses of broilers to LPS may reflect variability in the proportions or profiles of chemical mediators released during the ensuing inflammatory response. Broilers are more likely to exhibit profound pulmonary hypertension when LPS elicits the production of substantially more vasoconstrictors (TxA<sub>2</sub>, 5HT, PAF, ET-1, IL-6) than vasodilators (PGI<sub>2</sub>, NO). The pulmonary vasculature may appear hyporesponsive to LPS if substantially more vasodilators than vasoconstrictors are produced or if hyper-expression of iNOS causes NO to be generated in sufficient quantities to inhibit vasoconstrictor production or release. Additional research is needed to reveal key indices in the LPS-initiated inflammatory cascade that are correlated with the magnitude of the evoked pulmonary hypertensive response. These indices may prove useful in selecting broilers capable of thriving in spite of ongoing



**FIGURE 6.** Individual pulmonary arterial pressure (PAP) values for male broilers (mean  $\pm$  SEM,  $n = 8$ ) at the start of data collection (Start), at 5-min intervals during the control period (C5 and C10), within 30 s after injecting the volume control (VC), at 5-min intervals during the volume control period (VC5 and VC10), during the maximum PAP response within 90 s after the first injection (Tx1) of the thromboxane  $A_2$  mimetic U44069 (Tx1 Pk), at 5-min intervals after the first thromboxane mimetic injection (Tx5 and Tx10), within 30 s after endotoxin injection (E inj), at 5-min intervals after endotoxin injection (E5 to E30), during the maximum PAP response to endotoxin (E Pk), during the maximum PAP response within 90 s after the second injection (Tx2) of thromboxane mimetic (Tx2 Pk), and at 5-min intervals after the second thromboxane mimetic injection (Tx2 5 to Tx2 final). Different letters (a,b,c) designate differences between means over time ( $P \leq 0.05$ ) (adapted from Wideman et al., 2001).

exposure to aerosolized LPS, and the potential exists that similar criteria may be useful in selecting broilers for resistance to PHS. Uncovering the mechanisms responsible for the individually variable pulmonary hypertensive responsiveness to LPS will likely contribute to our understanding of the multi-factorial pathogenesis of PHS (Wideman, 2001). Our current knowledge of the roles of several relevant vasodilators and vasoconstrictors in broilers can be summarized as follows.

## Thromboxane

TxA<sub>2</sub>, whether administered i.v. as the potent TxA<sub>2</sub> mimetic U44069 or produced by circulating thrombocytes in response to bolus acid injections, increases the PVR and the PAP in broilers (Wideman et al., 1998a, 1999a, 2001). The TxA<sub>2</sub> mimetic U44069 triggered uniformly high pulmonary hypertension in broilers that subsequently exhibited a typically variable range of pulmonary vasoconstriction in response to LPS (Figure 6). The pulmonary hypertensive response to U44069 could also be elicited during the refractory or tolerant period when broilers become unresponsive to subsequent LPS injections (Figure 6). In contrast, the magnitude of the pulmonary vasoconstriction induced by bolus acid injections varied widely among individual broilers (Wideman et al., 1998a, 1999a, 2001). These observations suggest broilers vary relatively little in their pulmonary vascular respon-

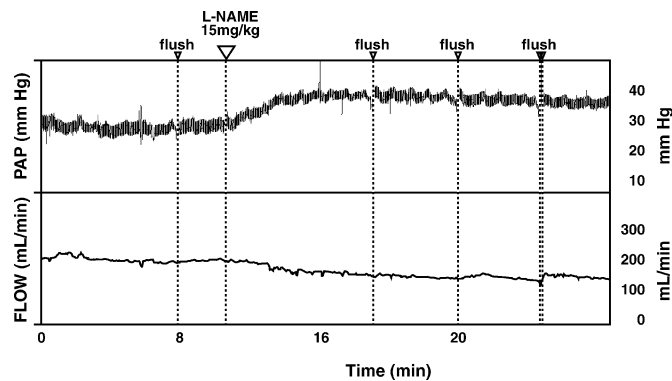
siveness to TxA<sub>2</sub>; nevertheless, innate factors responsible for initiating (e.g., ET-1), modulating (e.g., NO), or synthesizing (e.g., phospholipase A<sub>2</sub>, cyclooxygenase 2) TxA<sub>2</sub> may contribute to variability in the individual responsiveness to LPS (Wideman et al., 1998a, 2001; Martinez-Lemus et al., 1999; Villamor et al., 2002). For example, LPS-induced hyperexpression of the prostaglandin G/H 2 gene has been correlated with amplified TxA<sub>2</sub> production and pulmonary hypertension in rabbits (Conary et al., 1994; Delong et al., 1999).

## Serotonin

In domesticated avian species, biogenic amines (e.g., epinephrine, norepinephrine, phenylephrine, 5HT) can cause pulmonary vasoconstriction and thrombocyte aggregation and degranulation (Belamarich et al., 1968; Wideman, 1999; Villamor et al., 2002). 5HT is actively accumulated by mammalian platelets and avian thrombocytes and is released into the plasma during platelet/thrombocyte activation in response to PAF (Meyer and Sturkie, 1974; Cox, 1985; Lacoste-Eleau et al., 1994). 5HT is an extremely potent pulmonary vasoconstrictor capable of eliciting a sustained pulmonary hypertension when infused i.v. into broilers. In fact, 5HT is singularly the most potent pulmonary vasoconstrictor we have evaluated in broilers, capable of causing extensive pulmonary vasoconstriction leading to rapid, terminal suffocation unless injection dosages are carefully titrated to 100-fold lower than equivalently hypertensive doses in mammals (Chapman and Wideman, 2002). However, the accumulation of 5HT by thrombocytes, the in vivo release of 5HT, and plasma levels of 5HT have not been evaluated in broilers during the inflammatory response initiated by LPS. In humans, plasma 5HT levels increase markedly during gram-negative sepsis, and elevated plasma concentrations of 5HT have been implicated in the pulmonary hypertension associated with acute respiratory distress syndrome (Heffner and Repine, 1997). LPS triggers rapid intrapulmonary platelet aggregation and 5HT release in mice (Shibasaki et al., 1999). Elevated circulating levels of 5HT have been implicated in the initiation of acute and chronic pulmonary hypertension in several human and animal studies, including the pulmonary hypertensive response to appetite suppressant drugs (Seiler et al., 1974; Douglas et al., 1981; Herve et al., 1990; Brenot et al., 1993; Abenhaim et al., 1996; Weir et al., 1996; Egermayer et al., 1999; Kereveur et al., 2000). Polymorphisms of the 5HT membrane transporter or the transporter gene promoter may contribute to the susceptibility of mammals to pulmonary artery smooth muscle cell proliferation during the pathogenesis of pulmonary hypertension (Eddahibi et al., 2000; Simonneau et al., 2001).

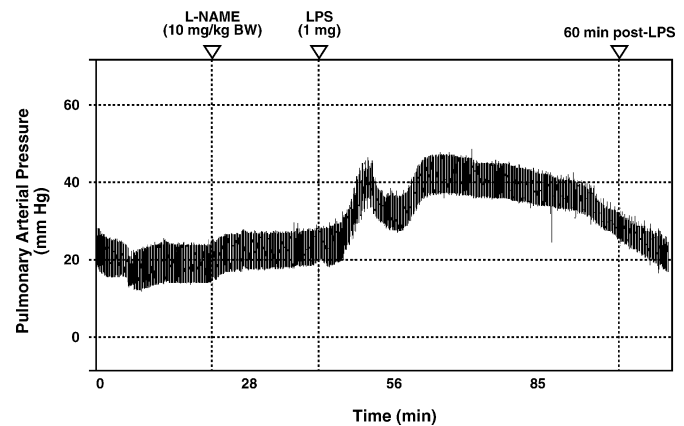
## Nitric Oxide

As summarized above, NO functions as a cytotoxic free radical (Gaston and Stamler, 1997; Bottje and Wideman, 1995), but it also plays an essential protective role that



**FIGURE 7.** Physiograph recording from an individual male broiler showing continuous values for pulmonary arterial pressure (PAP) and blood flow through the right pulmonary artery (FLOW) during an initial 10-min control period and after an i.v. injection of the nitric oxide synthase inhibitor  $N^G$ -nitro-L-arginine methyl ester (L-NAME) at 15 mg per kg BW to inhibit nitric oxide (NO) synthesis. Flush markers identify points where heparinized saline was injected to clear blood from the pulmonary arterial cannula. The moderate pulmonary hypertensive response to L-NAME developed rapidly and, as indicated by the trend toward a reduced FLOW, can be attributed to constriction of the pulmonary vasculature after inhibition of NO synthesis (R. F. Wideman and M. E. Chapman, unpublished).

is directly relevant to hemodynamic challenges (flow-dependent vasodilation) and pulmonary inflammatory responses (modulation of  $TxA_2$ , 5HT, and PAF release) (vide supra). LPS induces expression of the NOS gene in chickens (Chang et al., 1996), and different genetic lines of chickens can exhibit substantial innate variability in their LPS-mediated iNOS responsiveness coupled with corresponding variability in the levels of NO produced by their macrophages (Hussain and Qureshi, 1997). The characteristic level of LPS-induced iNOS expression in hyper- and hyporesponsive lines of chickens does not depend on the bacterial source of LPS, rather the magnitude of iNOS expression may be due to proportional macrophage expression of mCD14 and TLR4 receptors (Figures 3 and 4) (Hussain and Qureshi, 1997, 1998; Dil and Qureshi, 2002a,b; Qureshi, 2003). Pilot experiments were recently conducted to evaluate the modulatory role of NO using our standard protocol for assessing the pulmonary hypertensive response to LPS (Figures 7 and 8) (Wideman et al., 2001; Wang et al., 2002a,b). Broilers in the control group were injected with saline (volume/carrier vehicle control), and broilers in the L-NAME group were injected with 10 to 50 mg L-NAME/kg BW to block NO production. This dosage range for L-NAME has been demonstrated to be effective without causing acute toxicity in broilers (Grabarevic et al., 1997; Weidong et al., 2002). L-NAME inhibition of NO synthesis caused a rapid modest increase in PAP (Figure 7), confirming previous reports that tonic/basal NO synthesis promotes pulmonary vasodilation in chickens (Wideman et al., 1995; Wideman et al., 1996a; Villamor et al., 2002; Weidong et al., 2002). When both groups were injected with LPS, the L-NAME group exhibited an early pulmonary hypertensive peak that rarely develops in broilers in the absence of L-NAME (Wideman et al., 2001; Wang et al., 2002a), and that

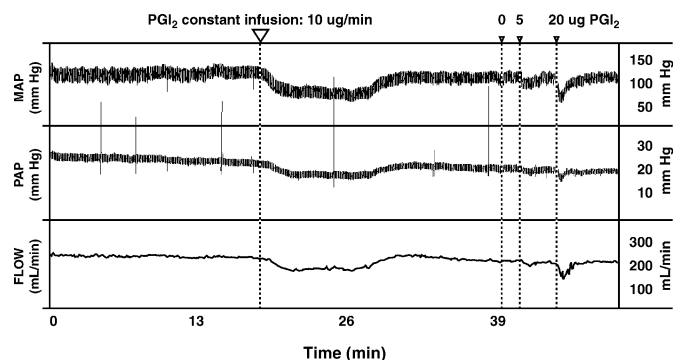


**FIGURE 8.** Physiograph recording from an individual male broiler showing continuous values for pulmonary arterial pressure (PAP) during an initial 15-min control period, for 10 min following an i.v. injection of the nitric oxide synthase inhibitor  $N^G$ -nitro-L-arginine methyl ester (L-NAME) at 10 mg/kg BW, and for > 60 min after an i.v. injection of 1 mg lipopolysaccharide (LPS). The L-NAME alone modestly increased the PAP; thereafter the response to LPS was biphasic, high in amplitude (peak > 40 mm Hg) and prolonged in duration (R. F. Wideman and M. E. Chapman, unpublished).

has been correlated with PAF-induced  $TxA_2$  synthesis in mammals (Snapper et al., 1998; Chlopicki et al., 1999; Wanecek et al., 2000; Gryglewski et al., 2001). The subsequent, more prolonged pulmonary hypertensive response to LPS was greater in duration in the L-NAME group when compared with the control group, and was higher in amplitude when the high dose of 50 mg of L-NAME/kg BW was used (Figure 8). In mammals, the delayed, more sustained pulmonary hypertensive response to LPS has been correlated with induction and transcription of the gene for pre-pro-endothelin, after which ET-1 constricts the pulmonary vasculature either directly via specific receptors on the smooth muscle cells or indirectly by stimulating leukocytes to produce  $TxA_2$  (Weitzberg, 1993; Faltin et al., 1996; Snapper et al., 1998; Wanecek et al., 2000). Chicken pulmonary artery rings exhibit dose-dependent contractions in response to ET-1, and NO attenuates this contractile response (Martinez-Lemus et al., 1999; Villamor et al., 2002). In this context, it is relevant that the capacity of i.v. injected LPS to elicit the delayed (sustained) phase of the pulmonary hypertensive response varies among mammalian species, and this variability appears to be positively correlated with species differences in the abundance of PIM available to promote  $TxA_2$  synthesis (Faltin et al., 1996). PIM are absent from avian lungs, and the pulmonary hypertensive response to LPS in broilers was not altered in magnitude or duration when an intrapulmonary inflammatory response (e.g., induced intrapulmonary monocyte/macrophage accumulation) was initiated 48 h prior to injecting LPS (Wang et al., 2002a, 2003).

### Prostacyclin

As shown in Figure 9, infusing  $PGI_2$  i.v. into clinically healthy broilers caused concurrent reductions in mean



**FIGURE 9.** Physiograph recording from an individual male broiler showing continuous values for mean systemic arterial pressure (MAP), pulmonary arterial pressure (PAP), and blood flow through the right pulmonary artery (FLOW). After an initial control period, prostacyclin ( $\text{PGI}_2$ ) was infused i.v. at  $10 \mu\text{g}/\text{min}$  for 10 min and then the  $\text{PGI}_2$  infusion ceased. Beginning approximately 40 min into the recording, the broiler was injected with 0 (volume control), 5, and  $20 \mu\text{g}$   $\text{PGI}_2$  i.v. to evaluate responses to different doses. In all cases,  $\text{PGI}_2$ -induced reductions in PAP coincided with reductions in MAP and FLOW (R. F. Wideman and M. E. Chapman, unpublished).

systemic arterial pressure, PAP, and CO. Preliminary analyses of these studies indicate that the reduction in CO represents the primary response to  $\text{PGI}_2$ , with the systemic and pulmonary hypertension primarily reflecting reduced flow rather than  $\text{PGI}_2$ -induced vasodilation (R. F. Wideman and M. E. Chapman, unpublished). These preliminary experiments were conducted on clinically healthy broilers having PAP values within the normal range. More dramatic vasodilation might be anticipated following  $\text{PGI}_2$  infusion into the pulmonary vasculature of broilers exhibiting pre-ascitic pulmonary hypertension or clinical PHS (Wideman and Tackett, 2000; Wideman et al., 2000).

## CONCLUSIONS

Broilers have a marginal pulmonary vascular capacity that renders them susceptible to the onset of pulmonary hypertension whenever any factor increases the PVR and forces the right ventricle to elevate the PAP to propel the CO through the lungs. The focal intrapulmonary inflammatory response to inhaled or bloodborne microparticulates and LPS triggers the release of a cascade of substances known to constrict the pulmonary vasculature. At the local tissue level, pulmonary vasoconstriction may serve to isolate and restrict the distribution of the offending antigen, permit inflammatory mediators and cytotoxic substances to accumulate in regionally high concentrations to neutralize the antigen, and shunt blood flow away from regions of the lungs where gas diffusion has been compromised by focal inflammatory damage to the endothelium and epithelium. These events apparently help preserve essential organ function (e.g., optimized blood-gas exchange) at the expense of subjecting focal tissues at the sites of inflammation to enhanced damage mediated by activated leukocytes. Problems arise from the scenario outlined above when the offending antigen

is widely distributed throughout the lungs, causing constriction of a significant proportion of the resistance vessels. The resulting increase in PVR constitutes the greatest threat when overlaid upon a preexisting incipient pulmonary vascular inadequacy, as in modern broilers. Innate mechanisms do exist to modulate life-threatening bouts of immune-mediated pulmonary hypertension. For example, following acute pulmonary vasoconstriction in response to LPS, a refractory or tolerant phase ensues, during which pulmonary hypertension cannot again be elicited even with very large doses of LPS. During this period of LPS tolerance, autocrine inflammatory stimuli (e.g., 5HT and  $\text{TxA}_2$  stimulation of additional 5HT and  $\text{TxA}_2$  release from platelets) presumably reach a crescendo and then subside. It remains to be determined whether broilers are more susceptible to invasion by gram-negative bacteria during the period of LPS tolerance. In addition, although NO itself clearly can have cytotoxic efficacy, NO generally is considered to modulate the tissue damage caused by activated leukocytes, modulate pro-inflammatory autocrine responses, and attenuate the pulmonary hypertension triggered by a disseminated pulmonary inflammatory response in mammals.

The results of pilot experiments provide evidence that in some broilers sufficient NO can be produced during an LPS challenge to modulate the production and biological impact of concurrently induced vasoconstrictors. In these individuals, pulmonary vasoconstriction in response to LPS is revealed only following pharmacological blockade of NOS with L-NAME. Blocking LPS-induced synthesis of NO caused higher-amplitude and longer-duration pulmonary hypertensive responses to LPS overall. These observations are consistent with the hypothesis that a pulmonary hypertensive response to LPS is not observed in some broilers because they innately generate more vasodilators (NO,  $\text{PGI}_2$ ) than vasoconstrictors ( $\text{TxA}_2$ , 5HT, PAF, IL-6, ET-1) during the LPS challenge. Variability among individuals in their pulmonary hypertensive responses to LPS clearly may reflect different innate characteristics of monocytes/macrophages in broilers (Chang et al., 1996; Hussain and Qureshi, 1997, 1998; Dil and Qureshi, 2002a,b). We now are poised to explore the possibility that LPS can serve as a relevant "tool" for evaluating the basis for pulmonary hypertension in broilers.

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# An Inadequate Pulmonary Vascular Capacity and Susceptibility to Pulmonary Arterial Hypertension in Broilers<sup>1</sup>

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**ABSTRACT** Broilers are susceptible to pulmonary hypertension syndrome (PHS; ascites syndrome) when their pulmonary vascular capacity is anatomically or functionally inadequate to accommodate the requisite cardiac output without an excessive elevation in pulmonary arterial pressure. The consequences of an inadequate pulmonary vascular capacity have been demonstrated experimentally and include elevated pulmonary vascular resistance (PVR) attributable to noncompliant, fully engorged vascular channels; sustained pulmonary arterial hypertension (PAH); systemic hypoxemia and hypercapnia; specific right ventricular hypertrophy, and right atrioventricular valve failure (regurgitation), leading to central venous hypertension and hepatic cirrhosis. Pulmonary vascular capacity is broadly defined to encompass anatomical constraints related to the compliance and effective volume of blood vessels, as well as functional limitations related to the tone (degree of constriction) maintained by the primary resistance vessels (arterioles) within the lungs. Surgical occlusion of 1 pulmonary artery halves the anatomical pulmonary vascular capacity, doubles the PVR, triggers PAH, eliminates PHS-susceptible broilers, and reveals PHS-resistant survivors whose lungs are innately capable of handling sustained increases in pulmo-

nary arterial pressure and cardiac output. We currently are using i.v. microparticle injections to increase the PVR and trigger PAH sufficient in magnitude to eliminate PHS-susceptible individuals while allowing PHS-resistant individuals to survive as progenitors of robust broiler lines. The microparticles obstruct pulmonary arterioles and cause local tissues and responding leukocytes to release vasoactive substances, including the vasodilator NO and the highly effective vasoconstrictors thromboxane A<sub>2</sub> and serotonin [5-hydroxytryptamine (5-HT)]. Nitric oxide is the principal vasodilator responsible for modulating (attenuating) the PAH response and ensuing mortality triggered by i.v. microparticle injections, whereas microparticle-induced increases in PVR can be attributed principally to 5-HT. Our observations support the hypothesis that susceptibility to PHS is a consequence of anatomically inadequate pulmonary vascular capacity combined with the functional predominance of the vasoconstrictor 5-HT over the vasodilator NO. The contribution of TxA<sub>2</sub> remains to be determined. Selecting broiler lines for resistance to PHS depends upon improving both anatomical and functional components of pulmonary vascular capacity.

**Key words:** pulmonary hypertension, broiler, ascites, nitric oxide, serotonin

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## INADEQUATE PULMONARY VASCULAR CAPACITY

Our research has confirmed that the pulmonary vascular capacity of modern broilers is marginally adequate to accommodate the cardiac output (CO) required to support the metabolic demands incurred by fast growth and the extremes of environmental temperatures (Wideman, 2000, 2001). The pulmonary vascular capacity can be

broadly defined to encompass metabolic limitations related to the tone (degree of contraction) maintained by the primary resistance vessels (pulmonary arterioles), as well as anatomical constraints related to the compliance and effective volume of the blood vessels (Figure 1; Wideman and Bottje, 1993). The pulmonary vasculature of broilers lacks functional elasticity (is marginally compliant) and is fully engorged with blood at a normal (resting) CO (Wideman and Kirby, 1995b; Wideman et al., 1996a,b). Consequently, the compensatory mechanisms known to minimize pulmonary vascular resistance (PVR) in mammals, such as arteriole dilation, capillary distention, and recruitment of previously underperfused vascular channels, appear to be minimally effective in broilers. Broilers possessing the most limited pulmonary vascular capacity develop pulmonary arterial hypertension (PAH), leading to terminal pulmonary hypertension syndrome (PHS;

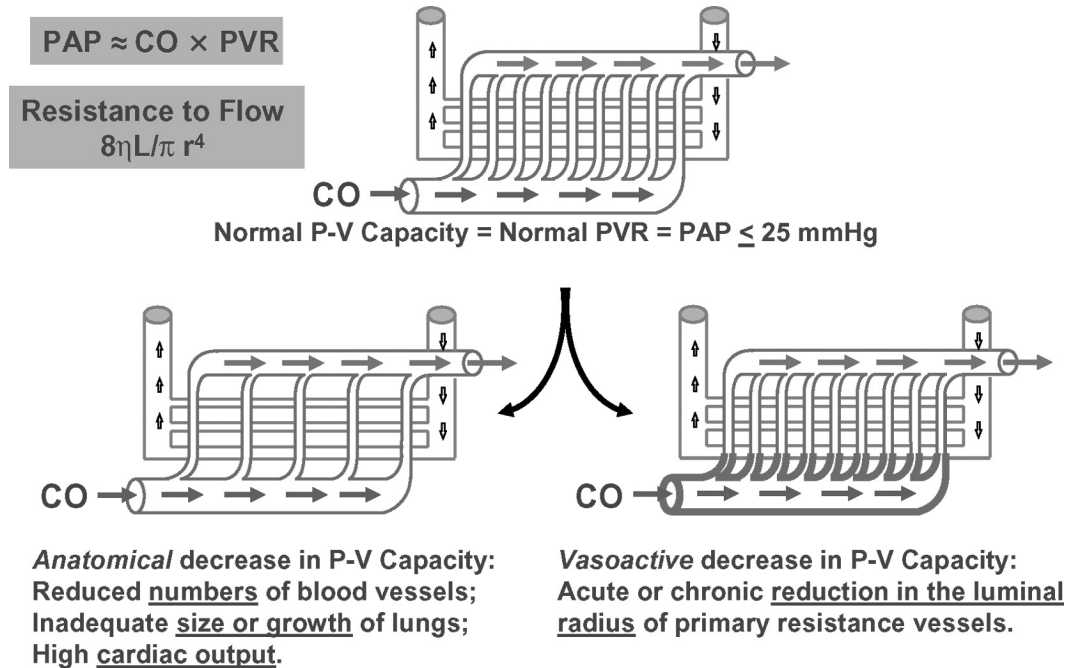
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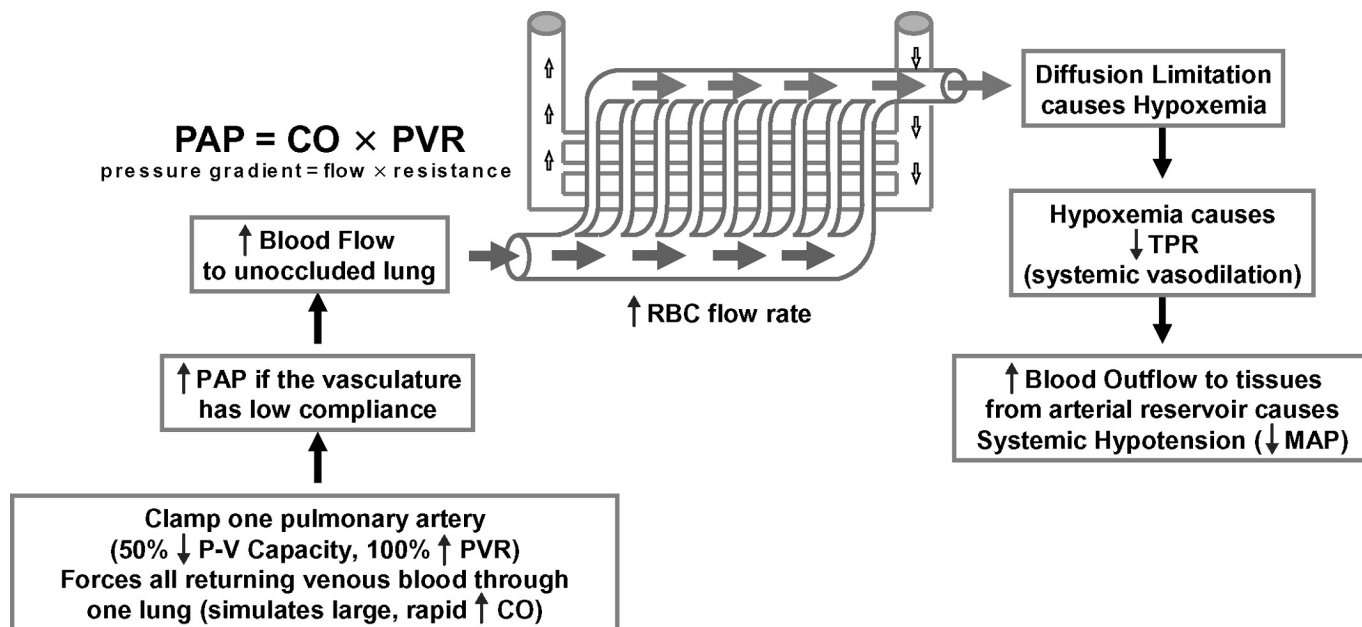
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**Figure 1.** The pulmonary vascular (P-V) capacity encompasses anatomical components such as the compliance (elasticity), volume, and cumulative cross-sectional radius of the blood vessels, as well as functional components including vascular responsiveness to vasoactive mediators affecting the tone maintained by the primary resistance vessels. Pulmonary arterial pressure (PAP) is (approximately) equal to the cardiac output (CO) multiplied by the pulmonary vascular resistance (PVR). Resistance to flow through blood vessels is principally determined by the vessels' radius ( $r^4$ ) rather than by length ( $L$ ) or the viscosity of blood ( $\eta$ ). Increases in PAP can be attributed to increases in CO, to anatomical inadequacies of pulmonary vascular capacity (increased PVR), or excessive vasoconstriction (increased PVR; adapted from Wideman and Bottje, 1993).

also known as ascites syndrome) when the right ventricle must develop an excessively elevated pulmonary arterial pressure (PAP) to propel the requisite CO through the lungs (Wideman and Bottje, 1993; Wideman, 2000, 2001; Wideman and Kirby, 1995a,b, 1996; Wideman et al., 1996a,b, 1997; Wideman and French, 1999). Higher PVR and CO values have been recorded in broilers with PAH than in clinically healthy individuals (Wideman and Tackett, 2000; Wideman et al., 2000; Lorenzoni and Wideman, unpublished data). Pulmonary arterial catheterization of apparently healthy broilers demonstrates that PAH precedes right ventricular hypertrophy (Wideman et al., 2006). Subsequent specific right ventricular "work hypertrophy" (increased mass of the free wall of the right ventricle) and elevated right ventricular weight-total ventricular weight (RV:TV) ratios consistently demonstrate the central role of pulmonary hypertension in the pathogenesis of PHS (Ploog, 1973; Cueva et al., 1974; Huchzermeyer and DeRuyck, 1986; Guthrie et al., 1987; Julian et al., 1987; Huchzermeyer et al., 1988; Julian, 1988, 1989, 1993; Lubritz et al., 1995; Owen et al., 1995a; Wideman and French, 1999; Wideman, 2000). Wedge pressures uniformly lower than 15 mmHg are obtained during pulmonary arterial catheterizations of broilers having PAP values ranging from 16 to 55 mmHg and corresponding RV:TV ranging from 0.20 to 0.51 (Chapman and Wideman, 2001; Wideman, 2001; Lorenzoni and Wideman, unpublished data). Wedge pressures  $\leq 15$  mmHg coupled with PAP  $\geq 25$  mmHg are specifically diagnostic for PAH attributable to elevated arteriole (precapillary) resistance.

In contrast, wedge pressures exceed 15 mmHg and increase in direct proportion to increases in PAP when pulmonary venous hypertension is triggered by elevated postcapillary (venous) resistance attributable to mitral valve insufficiency or congestive heart failure (Dawson and Linehan, 1997; Hermo-Weiler et al., 1998; Chapman and Wideman, 2001; Chemla et al., 2002; Deboeck et al., 2004; Benza and Tallaj, 2006). The crucial contribution of elevated precapillary resistance during the terminal pathogenesis of PHS can be deduced from consistent observations of medial muscle layer hypertrophy in the pulmonary arterioles of broilers developing clinical ascites (Cueva et al., 1974; Sillau and Montalvo, 1982; Huchzermeyer and DeRuyck, 1986; Hernandez, 1987; Julian, 1988; Peacock et al., 1989; Maxwell, 1991; Enkvetchakul et al., 1995; Xiang et al., 2002, 2004; Moreno de Sandino and Hernandez, 2003, 2006; Tan et al., 2005). Following the onset of PAH, the pathophysiological progression of PHS includes the gradual onset of systemic arterial hypoxemia (reduced partial pressure of  $O_2$  on arterial blood) and hypercapnia (elevated partial pressure of  $CO_2$  in arterial blood), polycythemia (increased hematocrit), reductions in total peripheral resistance and mean systemic arterial pressure, regurgitation by the monocuspid right atrioventricular valve, right-sided congestive heart failure, central venous hypertension, hepatic cirrhosis, and transudation of plasma from the surface of the liver into the abdominal cavity (ascites; Ploog, 1973; Wideman, 1984, 1988, 1999, 2000, 2001; Huchzermeyer and DeRuyck, 1986; Julian et al., 1987; Julian, 1988, 1993; Peacock et al., 1989, 1990;



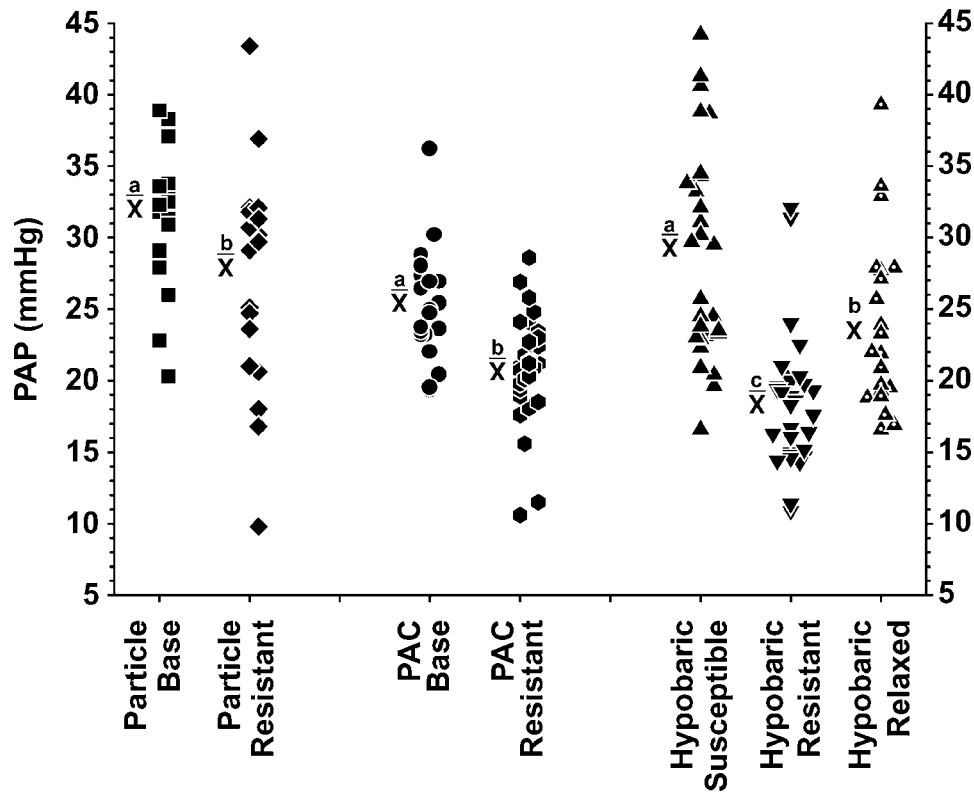
**Figure 2.** When the pulmonary vasculature is relatively noncompliant and fully engorged with blood, then experimentally reducing the pulmonary vascular (P-V) capacity by occluding 1 pulmonary artery doubles the pulmonary vascular resistance (PVR) and forces the right ventricle to double the pulmonary arterial pressure (PAP) to propel the entire cardiac output (CO) through the unoccluded lung. The rapid (within minutes) onset of systemic arterial hypoxemia (reduced partial pressure of O<sub>2</sub> on arterial blood) and hypercapnia (elevated partial pressure of CO<sub>2</sub> in arterial blood) are attributable to the onset of a diffusion limitation that is revealed when erythrocytes [red blood cells (RBC)] are forced to flow too rapidly past the pulmonary gas exchange surfaces to permit full blood-gas equilibration of O<sub>2</sub> and CO<sub>2</sub>. Hypoxemia dilates the systemic vascular resistance vessels, reducing total peripheral resistance (TPR) and thus the mean systemic arterial pressure (MAP; adapted from Wideman, 2001).

Julian and Mirsalimi, 1992; Wideman and Bottje, 1993; Fedde and Wideman, 1996; Forman and Wideman, 1999; Wideman et al., 1999b, 2000; Wideman and Tackett, 2000; Balog, 2003). The onset of hypoxemia and hypercapnia serve as reliable predictive indices that apparently healthy broilers will develop ascites (Peacock et al., 1989; Julian and Mirsalimi, 1992; Roush et al., 1996, 1997; Kirby et al., 1997; Wideman et al., 1998c, 2000). All major broiler genetics companies routinely use pulse oximetry to eliminate hypoxemic individuals from their pedigree lines and thereby markedly improve resistance to PHS. The spontaneous onset of hypoxemia and hypercapnia cannot be attributed to low atmospheric O<sub>2</sub> (hypoxia), poor circulation, anemia, intracardiac right to left shunts, hypoventilation, impaired respiratory function per se, or intrapulmonary vascular shunts through unventilated regions of the lungs. Instead, hypoxemia and hypercapnia are attributable to the onset of a diffusion limitation (West, 1993) that is revealed when erythrocytes are forced to flow too rapidly past the pulmonary gas exchange surfaces to permit full blood-gas equilibration of O<sub>2</sub> and CO<sub>2</sub> (Henry and Fedde, 1970; Peacock et al., 1989, 1990; Reeves et al., 1991; Wideman and Kirby, 1995a,b; Wideman et al., 1996a,b, 2000; Fedde et al., 1998; Forman and Wideman, 1999; Wideman and Tackett, 2000).

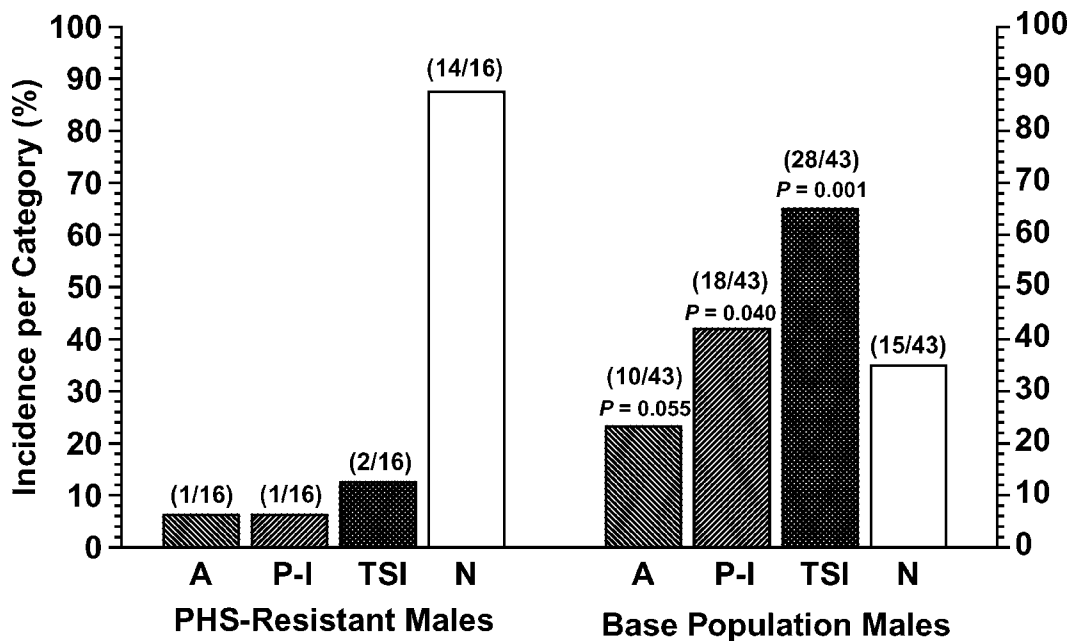
## REDUCING THE PULMONARY VASCULAR CAPACITY TRIGGERS PAH AND PHS

If an inadequate pulmonary vascular capacity is a primary determinant of susceptibility to PHS, then experi-

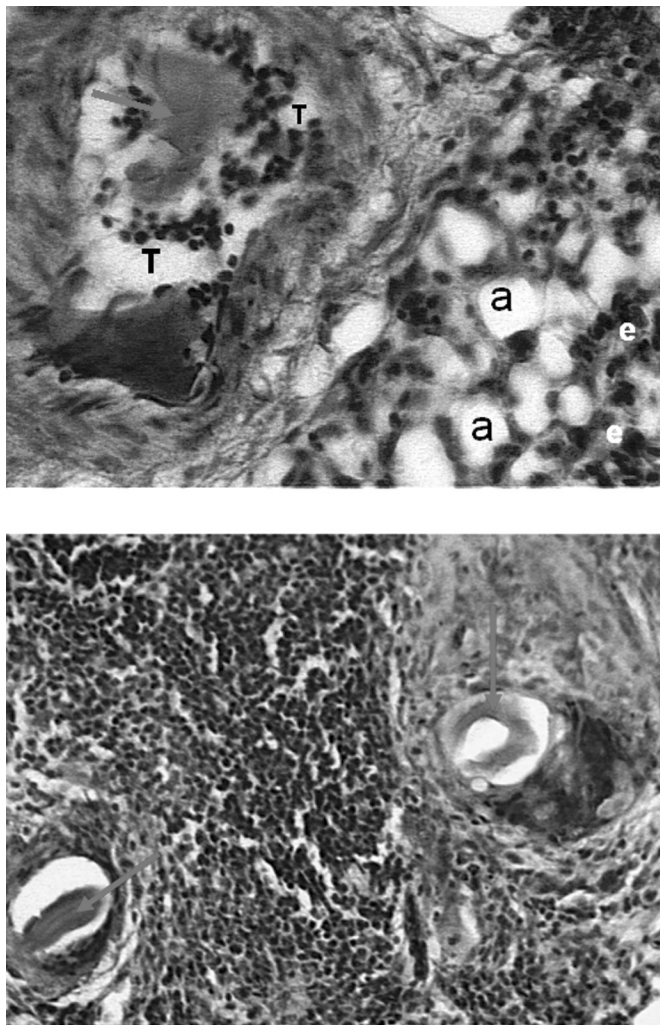
mentally reducing the pulmonary vascular capacity should initiate the pathophysiological progression leading to terminal PHS (Figure 2; Powell et al., 1985; Wideman and Bottje, 1993). Indeed, acutely tightening a snare to occlude 1 pulmonary artery halves the pulmonary vascular capacity, doubles the PVR, and forces the right ventricle to double the PAP to propel the entire CO through the unoccluded lung. Increasing the blood flow through 1 lung during acute unilateral pulmonary artery occlusion triggers a diffusion limitation resulting in the immediate onset of systemic arterial hypoxemia and hypercapnia. All of the responses to tightening a snare around 1 pulmonary artery are restored to their original baseline values within 5 min after the snare is released (Wideman and Kirby, 1995b; Wideman et al., 1996a,b, 1998a, 1999b; Forman and Wideman, 1999, 2001). Chronic unilateral pulmonary artery occlusion, accomplished by surgically obstructing 1 pulmonary artery with a silver clip, triggers the entire pathogenesis observed in broilers spontaneously developing PHS (Wideman and Kirby, 1995a, 1996; Wideman et al., 1997; Ruiz-Feria et al., 1999; Forman and Wideman, 2001). The survivors of chronic unilateral pulmonary artery occlusion possess a pulmonary vascular capacity sufficient to accommodate the combined challenges of an elevated PVR, a disproportionately high rate of blood flow through the unoccluded lung, and sustained PAH. Broiler breeders that thrive in spite of having 1 pulmonary artery occluded subsequently produce progeny exhibiting reduced PAP (Figure 3) and low RV:TV values combined with a cumulative 90% reduction in susceptibility to PHS when grown as rapidly as possible



**Figure 3.** Distributions of pulmonary arterial pressure (PAP) values obtained from individual male broilers from 7 lines: a base population (Particle Base) and a derivative line selected for 1 generation from the survivors of a 50% lethal dose microparticle injection (Particle Resistant); a second base population (PAC Base) and a derivative line selected for 3 generations using the unilateral pulmonary artery clamp technique (PAC Resistant); and susceptible, resistant, and relaxed lines selected for 10 generations under conditions of hypobaric hypoxia (Hypobaric Susceptible, Resistant, and Relaxed). The mean value for each line is indicated by X. Different letters (a,b,c) designate means that differed within respective selection techniques ( $P < 0.05$ ; Wideman et al., 2006; Bowen et al., 2006a).



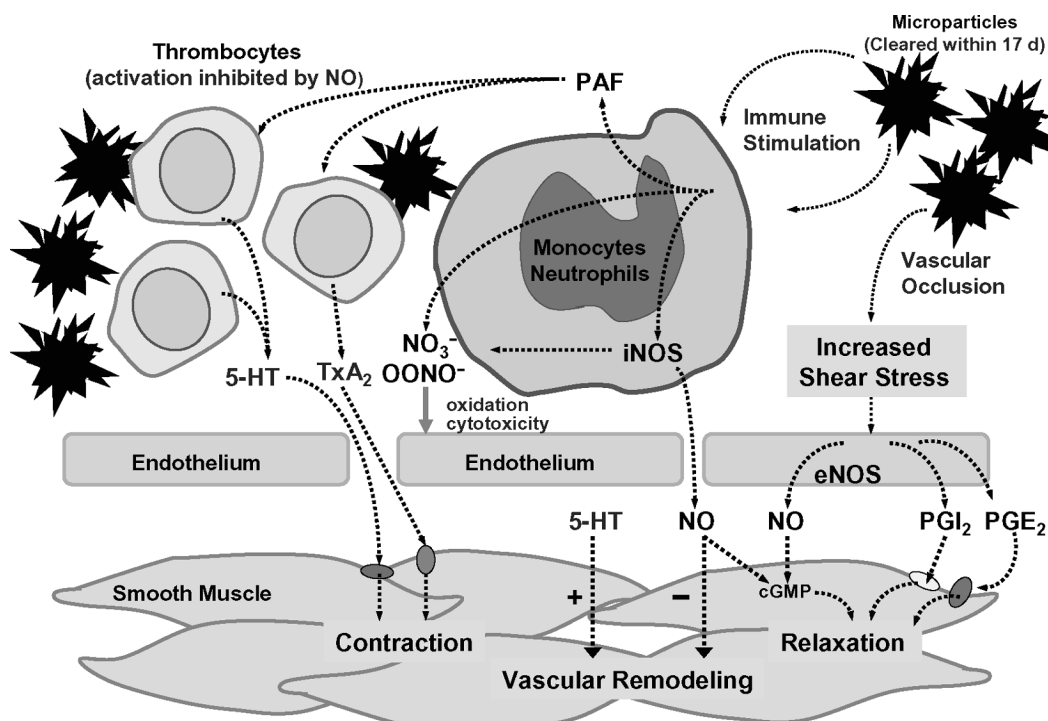
**Figure 4.** Male broilers from a base population (Base Population Males) and a derivative line selected for 2 generations using the unilateral pulmonary artery clamp technique (PHS-Resistant Males) were injected on d 20 with 0.4 mL of a cellulose microparticle suspension (0.02 g/mL) and then maintained at a thermoneutral temperature in an environmental chamber until d 49. Diagnostic categories include the following: ascites syndrome (A), 24 h postinjection mortality (P-I), total susceptibility index (TSI;  $TSI = A + P-I$ ), and nonascitic (N; normal, clinically healthy) through the end of the experiment. Numbers in parentheses reflect the affected/total evaluated; probability values are for interline comparisons within a diagnostic category (Wideman et al., 2002).



**Figure 5.** Intrapulmonary inflammatory response of male broilers injected i.v. at 22 d of age with 0.3 mL of cellulose microparticles. (Top panel) Hematoxylin and eosin staining of lung tissue obtained 20 min postinjection showing a cellulose microparticle (arrow) entrapped in a pulmonary arteriole surrounded by aggregating nucleated thrombocytes (T). Nucleated erythrocytes (e) are found in blood capillaries surrounding air (a) capillaries. (Bottom panel) Hematoxylin and eosin staining of lung tissue collected 48 h postinjection showing a lymphoid aggregate adjacent to 2 cellulose microparticles (arrows) within intraparenchymal arterioles. The occluded vessels are surrounded by granular tissue consisting primarily of macrophages, including giant cells, and fibrous tissue (Wideman et al., 2002; Wang et al., 2003).

during exposure to cool temperatures (Wideman and French, 1999, 2000; Chapman and Wideman, 2001; Wideman et al., 2006). This dramatic progress within 2 generations of selection demonstrates that selection pressure rigorously focused to challenge the pulmonary vascular capacity eliminates genes coding for a highly significant proportion of the PHS susceptibility in commercial broilers (Wideman, 2001). Our success has been achieved by identifying and then directly addressing the central pathogenic mechanisms expressed by preascitic (susceptible) broilers (e.g., PAH, hypoxemia, elevated RV:TV) instead of focusing on the downstream manifestations and pathologies expressed by chronically deteriorating individuals succumbing to clinical ascites.

Surgical occlusion of 1 pulmonary artery is impractical for large-scale genetic selection programs. Accordingly, we developed a technique in which microparticles having a size suitable for occluding precapillary arterioles are injected into a systemic vein to be carried to the lungs by the returning venous blood [US patent 6,720,473 protects the exclusive rights of the University of Arkansas to all uses of the i.v. microparticle injection technology within the context of evaluating or affecting the pulmonary vascular capacity, pulmonary vascular resistance, pulmonary hypertension, cardiopulmonary hemodynamics, and susceptibility to PHS (ascites) in domesticated animal species]. Pulmonary arterioles become occluded in proportion to the number and size of the microparticles injected, thereby increasing the PVR and triggering acute physiological responses that mirror those previously observed following acute unilateral pulmonary artery occlusion and that are characteristic of preascitic broilers (Wideman and Erf, 2002; Wideman et al., 2005b, 2006). After injecting a suitable dose of microparticles, broilers with the most limited pulmonary vascular capacity rapidly succumb to respiratory insufficiency (acute postinjection mortality); those with a marginal pulmonary vascular capacity develop hypoxemia, hypercapnia, and PAH, leading to terminal PHS; and those having a sufficiently robust pulmonary vascular capacity thrive as clinically healthy and resistant survivors (Figure 4; Wideman et al., 2002). The combined mortality (acute postinjection + ascites mortality) is proportional to the quantity of microparticles injected and the magnitude of the resulting sustained pulmonary hypertensive response. Broiler lines selected for PHS resistance are substantially more resistant to microparticle injections when compared with their respective unselected (base) populations or PHS-susceptible lines (Figure 4; Wideman et al., 2002). Microparticle injections currently are being used to select commercial broiler lines having a robust pulmonary vascular capacity and improved resistance to PHS. For every commercial and experimental population evaluated to date, broilers from lines known to be resistant to the spontaneous onset of PHS exhibit significantly lower mortality when injected i.v. with microparticle doses that trigger high mortality among broilers from susceptible lines. Breeder parent survivors of microparticle injections produce progeny exhibiting reduced PAP (Figure 3) and RV:TV values as well as reduced susceptibility to PHS when challenged with cool temperature exposure (Wideman et al., 2002, 2006; Wideman, unpublished data). Microparticle injections also can be used to select broilers whose robust cardiopulmonary capacity confers improved growth and livability during exposure to heat stress (Wideman et al., 2003a), thereby reemphasizing the requirement for the lungs to accommodate the increases in CO necessary to deliver additional O<sub>2</sub> to the tissues whenever broilers are subjected to nonthermoneutral temperatures (Wideman, 1999, 2000).



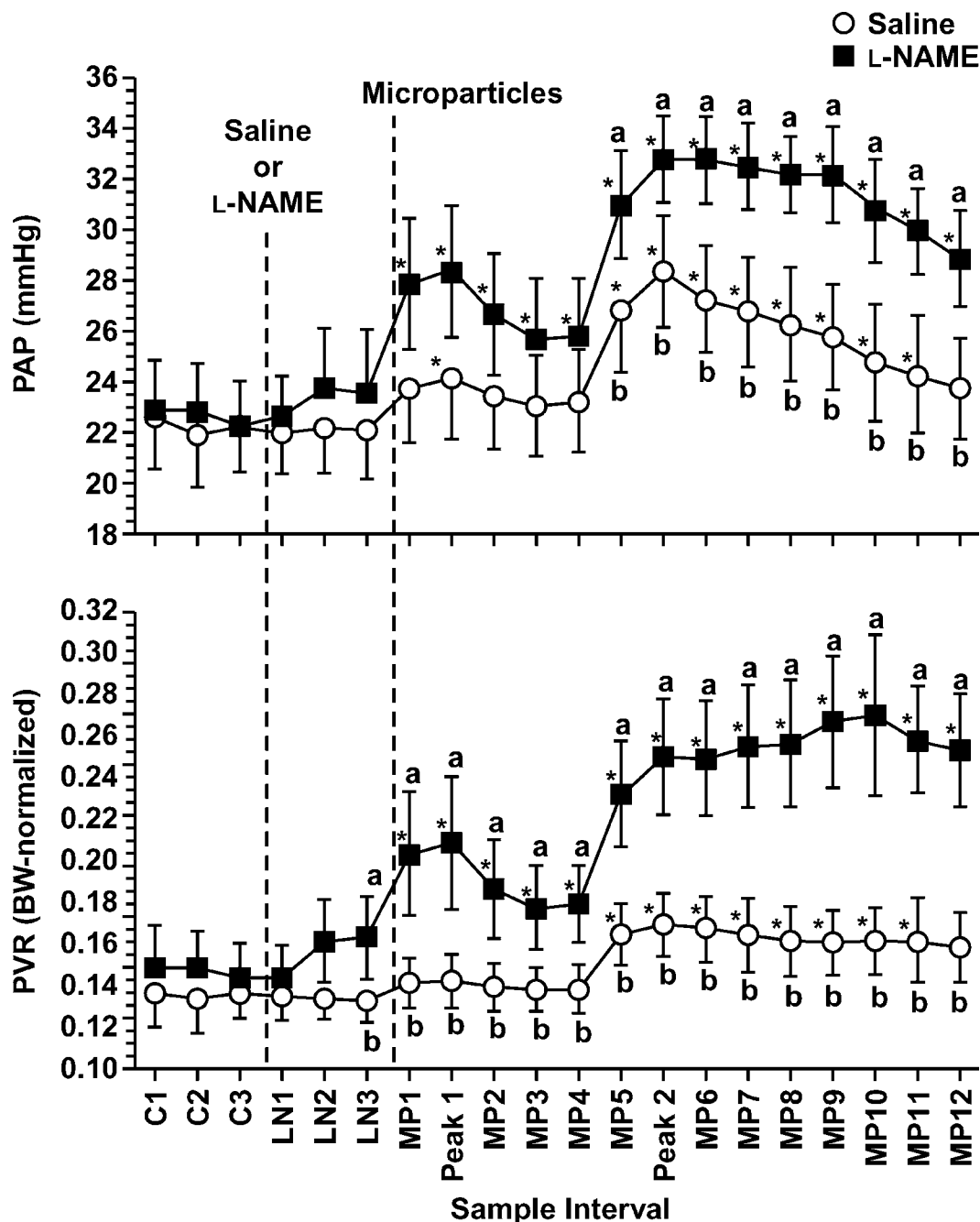
**Figure 6.** Microparticle occlusion of pulmonary arterioles increases blood flow and shear stress through unoccluded channels, with the resulting increase in shear stress activating endothelial NO synthase (eNOS) to produce the potent vasodilator NO as well as the putative eicosanoid vasodilators prostacyclin (PGI<sub>2</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Entrapped microparticles activate monocytes and macrophages, triggering a cascade of intracellular signaling events including the release of platelet-activating factor (PAF) and expression of inducible NO synthase (iNOS). Entrapped microparticles and PAF stimulate thrombocytes to release the pulmonary vasoconstrictors thromboxane (TxA<sub>2</sub>) and serotonin [5-hydroxytryptamine (5-HT)]. The iNOS enzyme produces copious quantities of NO and derivative reactive O<sup>2</sup>-N species [e.g., nitrite (NO<sub>3</sub><sup>-</sup>) and peroxynitrite (OONO<sup>-</sup>)] that are nonspecifically cytotoxic. Nitric oxide relaxes pulmonary vascular smooth muscle, NO modulates (inhibits) PAF activation of thrombocytes and the release of TxA<sub>2</sub> and 5-HT, and NO and PGI<sub>2</sub> inhibit platelet aggregation and the formation of obstructive microthrombi. Vascular remodeling (hypertrophy, hyperplasia, and distal extension of pulmonary arteriole smooth muscle cells) is inhibited by NO (Tan et al., 2005), whereas 5-HT stimulates vascular remodeling. cGMP = cyclic guanosine monophosphate (adapted from Wideman et al., 2004).

## IMMUNE-MEDIATED VASODILATION AND VASOCONSTRICTION

### Role of NO

Physical occlusion of precapillary arterioles is not the only mechanism by which microparticles can influence the pulmonary vascular resistance. Within minutes after being injected, the entrapped microparticles are surrounded by focal aggregates of thrombocytes and by monocytes and macrophages infiltrating the perivascular region. Within 24 to 48 h, lymphoid aggregates form around occluded vessels (Figure 5; Wideman et al., 2002; Wang et al., 2003). This dynamic intrapulmonary inflammatory response potentially can trigger the leukocytes and adjacent vascular endothelium to synthesize and release potent vasoactive compounds near the vascular smooth muscle (Figure 6; Wideman, 2001; Wideman et al., 2004). Of specific interest is the synthesis of NO by the enzyme NO synthase (NOS), which is constitutively expressed in vascular endothelial cells [endothelial NOS (eNOS) or NOS-3] or is induced in activated monocytes and macrophages [inducible NOS (iNOS) or NOS-2; Chang et al., 1996; Hussain and Qureshi, 1997, 1998; Dil and Qureshi, 2002a,b; Qureshi, 2003]. In chickens, NO dilates the pulmonary vasculature and attenuates (modu-

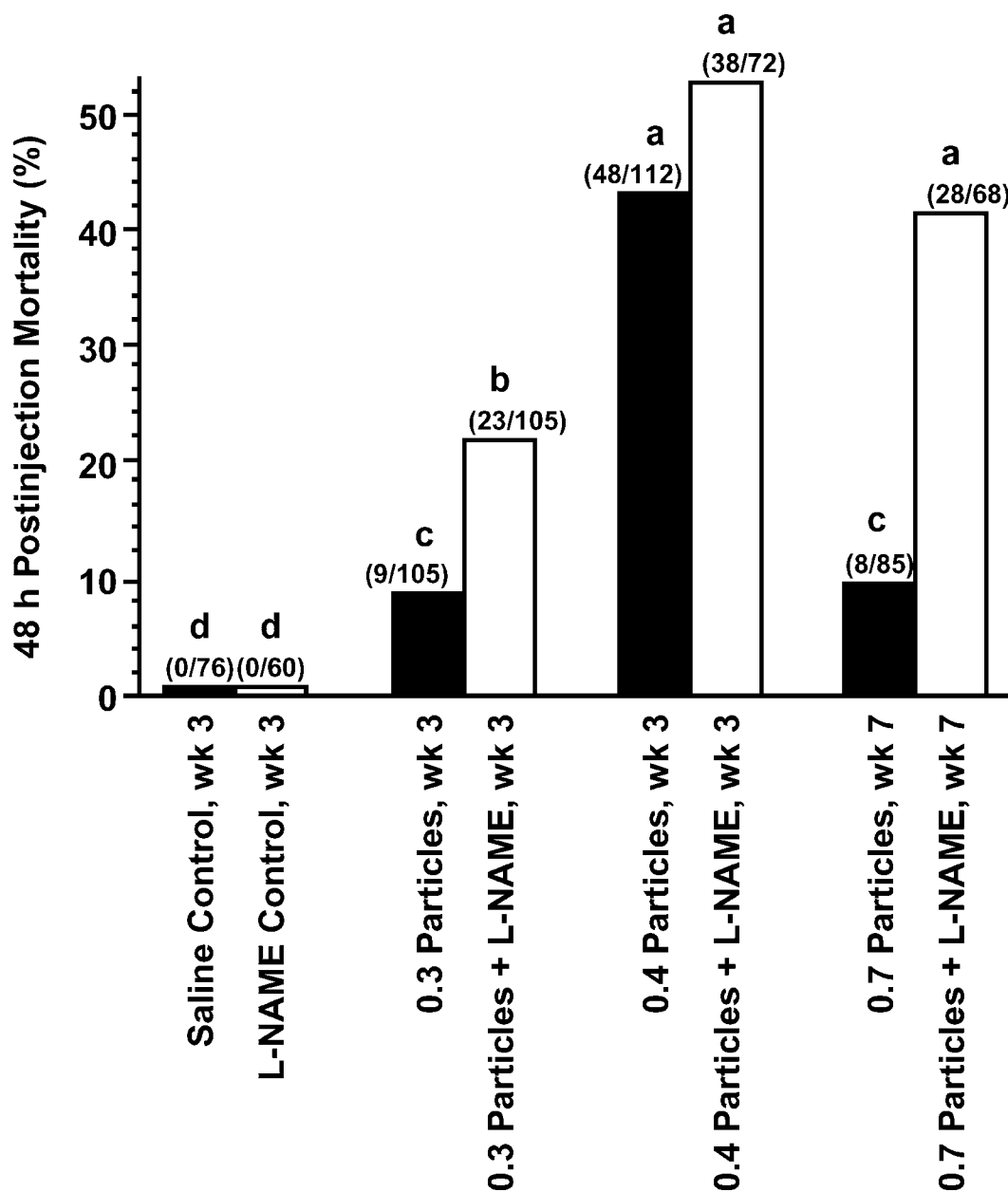
lates) the production, release, and vascular responsiveness to vasoconstrictors (Figure 6). When both eNOS and iNOS are inhibited by N<sup>ω</sup>-nitro-L-Arg methyl ester (L-NAME), the ensuing reduction in NO synthesis leads to pulmonary arterial vasoconstriction, PAH, and PHS (Wideman et al., 1995, 1996a, 1998a, 2004; Grabarevic et al., 1997; Martinez-Lemus et al., 1999, 2003; Ruiz-Feria et al., 2001; Villamor et al., 2002; Wang et al., 2002c; Weidong et al., 2002; Odom et al., 2004; Wideman and Chapman, 2004). Pretreating broilers with L-NAME doubles the increases in PAP and PVR elicited by subsequent microparticle injections (Figure 7). Similarly, the mortality triggered within 48 h after injecting microparticles more than doubles when L-NAME is combined with microparticle injection doses that otherwise cause relatively low mortality in the absence of L-NAME (Figure 8; Wideman et al., 2005b). The magnitude and duration of the microparticle-induced systemic arterial hypoxemia remains unaffected by L-NAME, indicating that hypoxemia per se contributes minimally to PAH and postinjection mortality (Wideman et al., 2005b), replicating previous evidence that PAH and PHS attributable to unilateral pulmonary artery or bronchus occlusion are not directly attributable to hypoxemia and hypercapnia per se (Wideman et al., 1996b, 1997). Pretreating broilers with the selective iNOS inhibitor aminoguanidine marginally amplifies the increase in



**Figure 7.** Pulmonary arterial pressure (PAP) and relative pulmonary vascular resistance (PVR) for male broilers pretreated with saline (control) or the NO synthase inhibitor N<sup>ω</sup>-nitro-L-Arg methyl ester (L-NAME) followed by i.v. injections of cellulose microparticles (mean  $\pm$  SEM,  $n = 12$ /group). Data were averaged electronically during representative sample intervals at 4, 2, and 0.5 min before injecting saline or L-NAME (control sample intervals C1, C2, and C3, respectively); at 0.5, 2.5, and 5 min after injecting saline or L-NAME (L-NAME sample intervals LN1, LN2, and LN3, respectively); at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 15, 20, 25, and 30 min after the cellulose microparticle injection (sample intervals MP1 through MP12, respectively); and during the peak PAP responses that occurred within 90 s (peak 1) and 4 to 6 min (peak 2) after the microparticle injection. Asterisks (\*) denote values that differ from the preinjection values (C1, C2, C3) within a group ( $P \leq 0.05$ ). Different letters (a,b) designate group means that differed within a time interval ( $P \leq 0.05$ ; Wideman et al., 2005b).

PAP elicited by microparticle injections in progeny from the survivors of a 50% lethal dose microparticle selection (Figure 9), but not in progeny from survivors of unilateral pulmonary artery occlusion (Wideman et al., 2006). Expression of iNOS by activated monocytes and macrophages responding to microparticles entrapped in the lungs requires hours rather than minutes (Hamal et al., 2006). The levels of NO produced in response to micropar-

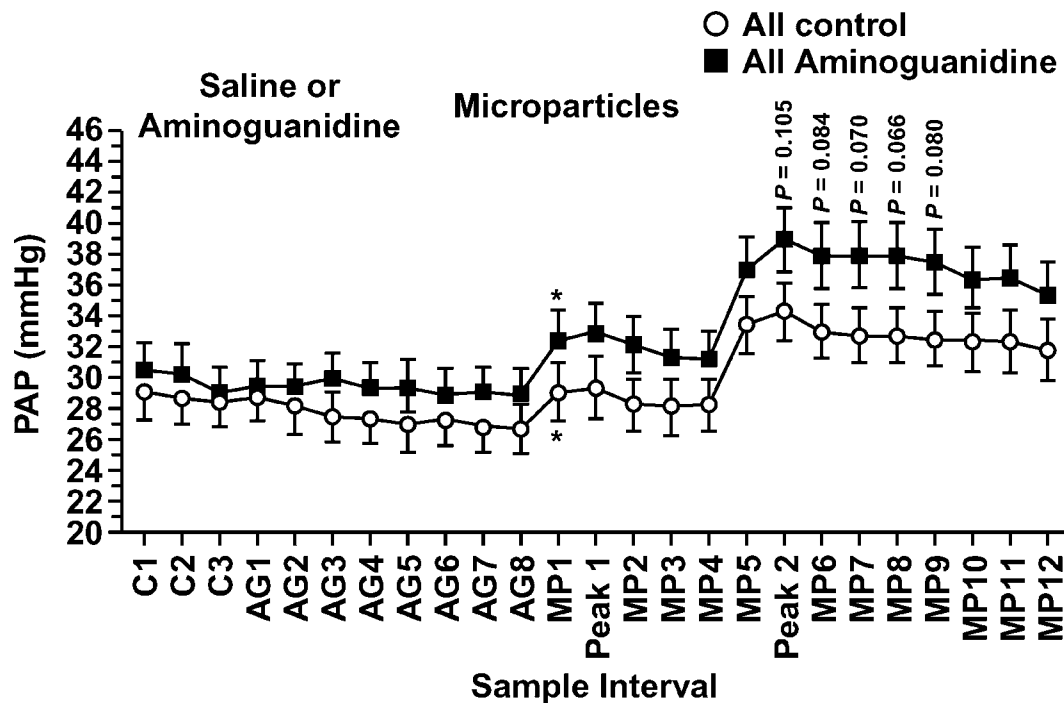
ticle entrapment are sufficient to elicit local vasodilation (Wideman et al., 2005b, 2006) but insufficient to elevate total NO concentrations in the systemic circulation (Bowen et al., 2006b). Indeed, when the combined processes of NO dilution in extracellular fluid, NO binding to hemoglobin, NO exhalation as a gas, and rapid renal clearance are taken into consideration, it becomes evident that low levels of NO capable of effectively relaxing vas-



**Figure 8.** Mortality percentages for male broilers injected at 3 wk of age with the following: saline (Saline Control);  $N^G$ -nitro-L-Arg methyl ester (L-NAME Control); 0.3 mL of microparticles (0.3 Particles); 0.3 mL of microparticles and L-NAME (0.3 Particles + L-NAME); 0.4 mL of microparticles (0.4 Particles); or 0.4 mL of microparticles and L-NAME (0.4 Particles + L-NAME). Male broilers also were injected at 7 wk of age with 0.70 mL of microparticles alone (0.7 Particles) or in combination with L-NAME (0.7 Particles + L-NAME). Different letters (a,b,c,d) designate mortality incidences that differed ( $P \leq 0.05$ ) among groups (Wideman et al., 2005b).

cular smooth muscle need not significantly elevate total plasma NO concentrations (Chapman and Wideman, 2006a). The current evidence indicates that NO, generated acutely by eNOS and subsequently supplemented when iNOS is expressed, performs dual roles as a vasodilator and a modulator of the production, release and vascular responsiveness to vasoconstrictors (Figure 6; Wideman et al., 2002, 2004, 2005b, 2006). Broiler lines undergoing selection for improved resistance to PHS should be monitored for potential coselection of eNOS and iNOS expression. For example, broilers are most likely to survive mi-

croparticle injections if their endothelial cells express more eNOS and if their leukocytes possess inflammatory response profiles that have been shifted toward enhanced recognition and removal activity (more rapid clearance of particles from the vasculature), enhanced vasodilator production (e.g., increased iNOS expression), and attenuated vasoconstrictor production (Wideman et al., 2004, 2005b). Reduced pulmonary arteriole eNOS expression has been reported in broilers developing PHS during chronic exposure to hypobaric hypoxia (Moreno de Sandino and Hernandez, 2003, 2006), but pulmonary eNOS



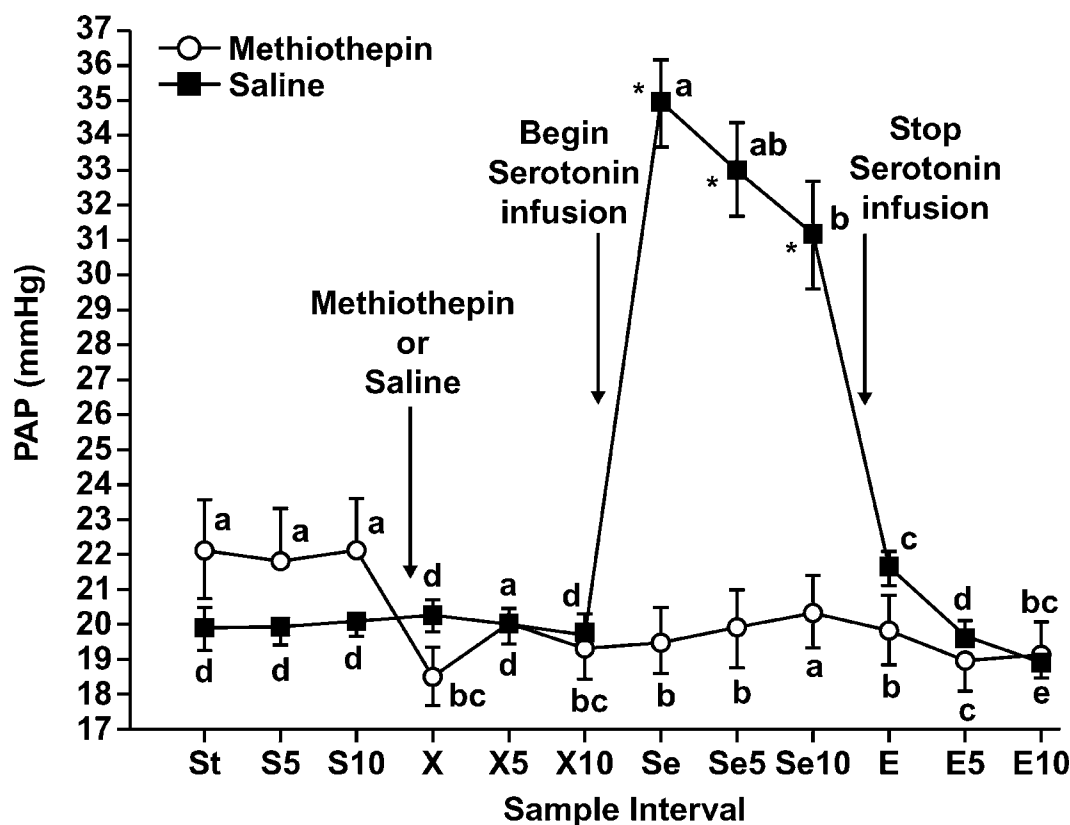
**Figure 9.** Pulmonary arterial pressures (PAP) are shown for progeny from the survivors of a 50% lethal dose microparticle selection pretreated with saline (control) or aminoguanidine followed by i.v. injections of cellulose microparticles. Data were averaged electronically during representative sample intervals at 4, 2, and 0.5 min before injecting saline or aminoguanidine (sample intervals C1, C2, and C3); within 0.5 min after injecting saline or aminoguanidine (AG1) and at 2-min intervals throughout the subsequent 15 min (sample intervals AG2 to AG8); within 0.5, 1, 1.5, 2, 4, 6, 8, 10, 15, 20, 25, and 30 min after injecting cellulose microparticles (sample intervals MP1 through MP12, respectively); and during the minor and major peak responses (peaks 1 and 2; mean  $\pm$  SEM,  $n = 18$  per group). Asterisks (\*) denote the earliest postinjection values that were higher than the respective preinjection values (C3 vs. AG1 to AG8, or AG8 vs. MP1 to MP12) within a group ( $P \leq 0.05$ ). Probability values that approached but did not attain significance at  $P \leq 0.05$  also are indicated (Wideman et al., 2006).

and iNOS expression levels have not been associated with the onset of PHS induced by chronic exposure to subthermoneutral temperatures (Teshfam et al., 2006).

### Role of Serotonin (5-Hydroxytryptamine)

Nucleated avian thrombocytes are the most numerous leukocytes in avian blood and are functional homologs of mammalian platelets. Thrombocytes accumulate serotonin [5-hydroxytryptamine (5-HT)] within intracellular storage granules that are released upon thrombocyte activation and aggregation (Inouye et al., 1969; Kimura, 1969; Kuruma et al., 1970; Simoneit et al., 1970; Sorimachi et al., 1970, 1974; Meyer and Sturkie, 1974; Cox, 1985; Lacoste-Eleaume et al., 1994). Serotonin autostimulates thrombocyte activation and aggregation (Belamarich et al., 1968; Belamarich and Simoneit, 1973), and activated avian thrombocytes are phagocytic toward microparticulates and bacteria (Glick et al., 1964; Carlson et al., 1968; Sterz and Weiss, 1973; Chang and Hamilton, 1979a,b; Awadhiya et al., 1980; Ohata and Ito, 1986; Lam, 1997; DaMatta et al., 1998; Roland and Birrenkott, 1998; Wigley et al., 1999). Thrombocytes rapidly surround microparticles entrapped in intimate proximity to pulmonary arteriole smooth muscle, providing an ideal milieu in which the vasoconstrictors 5-HT and thromboxane  $A_2$  (Tx $A_2$ ) can further amplify increases in PVR caused by physical occlusion (Figures 5 and 6). Serotonin increases the PVR

and PAP in broilers and is singularly the most potent pulmonary vasoconstrictor we have evaluated. Serotonin is capable of triggering essentially instantaneous and fully obstructive vasoconstriction, leading to an immediate >90% reduction in CO and terminal suffocation within 30 s in clinically healthy broilers unless i.v. infusion rates are carefully titrated to at least 10-fold lower than levels typically used to elicit PAH in mammals (Chapman and Wideman, 2002). The pulmonary hemodynamic responses to 5-HT recently were evaluated in broilers pretreated with the selective 5-HT $_{2A}$  receptor antagonist ketanserin or with the nonselective 5-HT $_{1/2}$  receptor antagonist methiothepin. Ketanserin has high affinity for the 5-HT $_{2A}$  receptor but also binds less potently to the 5-HT $_{2C}$ , 5-HT $_{2B}$ , 5-HT $_{1D}$ , adrenergic, and dopamine receptors (Barnes and Sharp, 1999). Methiothepin is a nonselective 5-HT $_{1}$  and 5-HT $_{2}$ , as well as a 5-HT $_{5.7}$  receptor antagonist with varying degrees of selectivity; however, it displays high affinities for 5-HT $_{1A}$  and 5-HT $_{1B}$  receptor subtypes in rats (Engel et al., 1986). Pretreating broilers with ketanserin failed to alter the PAH response to subsequent 5-HT infusion, whereas pretreatment with methiothepin reduced PAP below baseline values and virtually eliminated increases in PVR and PAP elicited by 5-HT (Figure 10). Methiothepin clearly blocked 5-HT-mediated increases in PVR and PAP in broilers, although the specific receptor subtype involved remains to be determined (Chapman and Wideman, 2006b). In a subsequent study

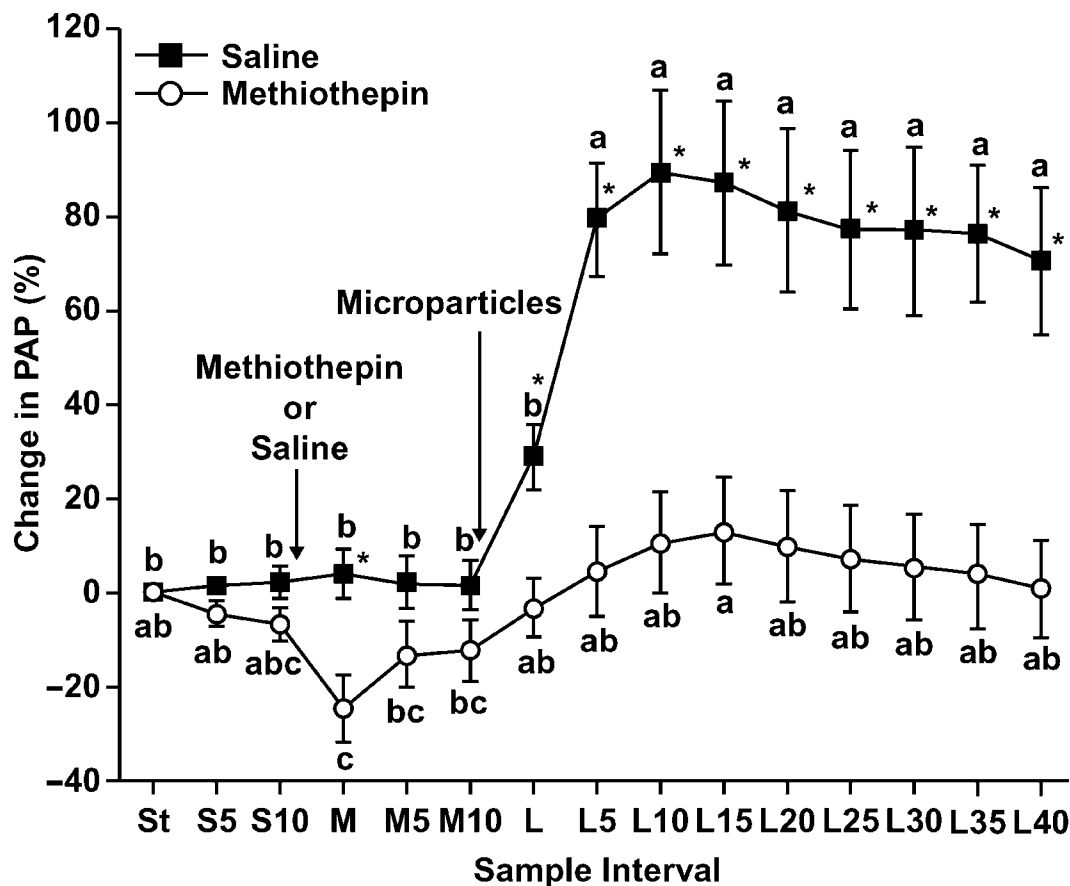


**Figure 10.** Pulmonary arterial pressure (PAP) in male broilers pretreated with saline (control) or the serotonin receptor inhibitor methiothepin, followed by i.v. infusion of serotonin. Data were averaged electronically during representative sample intervals at the start of data collection and at 5-min intervals thereafter (St, S5, S10); within 0.5 min after injecting saline or methiothepin and at 5-min intervals thereafter (X, X5, X10); within 0.5 min after the beginning of serotonin infusion and at 5-min intervals thereafter (Se, Se5, Se10); and within 5 min after stopping the serotonin infusion and at 5-min intervals thereafter (E, E5, E10; mean  $\pm$  SEM,  $n = 20$  per group). Asterisks (\*) designate group means that differed within a sample interval ( $P \leq 0.05$ ). Different letters (a,b,c,d,e) designate values that differed within a group across sample intervals ( $P \leq 0.05$ ; Chapman and Wideman, 2006a).

by Chapman and Wideman (2006c), methiothepin was used to evaluate the role of 5-HT in the onset of PAH triggered by i.v. microparticle or lipopolysaccharide injections. Pretreatment with methiothepin reduced PAP below baseline values, demonstrating that 5-HT likely exerts tonic control of PVR. Microparticle injections increased PAP by 90% within 10 min in untreated (control) broilers, but the same dose of microparticles failed to significantly elevate PAP in broilers that were pretreated with methiothepin (Figure 11). Injecting a high dose of microparticles (1.0 mL of 0.02 g/mL) into broilers from a PHS-susceptible line (Anthony et al., 2001) elicited 78% mortality in untreated controls as compared with only 20% in those pretreated with methiothepin (Chapman and Wideman, 2006c). Injecting the same microparticle dose into a PHS-resistant line (Anthony et al., 2001) elicited 12% mortality in untreated controls as compared with zero mortality in those pretreated with methiothepin (Chapman and Wideman, 2006c). Methiothepin had minimal effect on lipopolysaccharide-mediated PAH (Chapman and Wideman, 2006c). All available evidence supports the hypothesis that 5-HT plays a key role in maintaining the basal tone of the pulmonary vasculature and a dominant role in the increases in PVR and PAP elicited by microparticle injections (Chapman and Wideman, 2006b,c).

## CONCLUSIONS

Selecting broiler lines for improved resistance to PHS currently appears to depend upon improving both anatomical and functional components of pulmonary vascular capacity. Our observations are consistent with the model illustrated in Figure 6. We have confirmed that sufficient NO can be produced (initially by eNOS but subsequently supplemented by iNOS over prolonged periods) during pulmonary inflammatory challenges to perform the dual roles of dilating the pulmonary vasculature as well as modulating the production, biological effect, or both, of concurrently induced vasoconstrictors (Wideman and Chapman, 2004; Wideman et al., 2005b, 2006; Bowen et al., 2006a,c; Chapman and Wideman, 2006a). We do not know whether NO accomplishes its principal protective role by dilating the pulmonary arterioles or by modulating the release of vasoconstrictors by thrombocytes. The eicosanoid vasodilators prostacyclin ( $\text{PGI}_2$ ) and prostaglandin  $\text{E}_2$  do not reduce PVR when infused i.v. into clinically healthy broilers, broilers whose pulmonary vasculature has been precontracted with arachidonic acid, or broilers with preexisting PAH (Wideman et al., 2005a, unpublished data). Accordingly,  $\text{PGI}_2$  and prostaglandin  $\text{E}_2$  do not appear to dilate the pulmonary vasculature in



**Figure 11.** Percentage change in pulmonary arterial pressure (PAP) in male broilers pretreated with saline (control) or the serotonin receptor inhibitor methiothepin, followed by i.v. injections of cellulose microparticles. Data were averaged electronically during representative sample intervals at the start of data collection and at 5-min intervals thereafter (St, S5, S10); within 0.5 min after injecting saline or methiothepin and at 5-min intervals thereafter (M, M5, M10); and within 0.5 min after microparticle injection and at 5-min intervals thereafter (L to L40; mean  $\pm$  SEM,  $n = 15$ /group). Asterisks (\*) denote values that were higher than the respective preinjection (St to S10) values within a group ( $P \leq 0.05$ ). Different letters (a,b,c) designate group means that differed within a sample interval ( $P \leq 0.05$ ; Chapman and Wideman, 2006b).

broilers, although  $\text{PGI}_2$  may significantly modulate thrombocyte activation (Wideman et al., 2004, 2005a). Thromboxane  $\text{A}_2$ , whether administered i.v. as the potent  $\text{TxA}_2$  mimetic U44069 or produced by circulating thrombocytes, consistently causes pulmonary vasoconstriction and PAH in broilers. The precise contribution of  $\text{TxA}_2$  to increases in PVR in PHS-susceptible broilers remains to be determined (Wideman et al., 1998b, 1999a, 2001, 2004, 2005a; Chapman and Wideman, 2006b). Serotonin clearly plays a key role in increasing the basal tone (partial state of contracture) of the pulmonary resistance vessels and a dominant role in microparticle-induced increases in PVR and PAP in broilers (Chapman and Wideman, 2002, 2006b,c). It currently appears likely that microparticle selection serves to eliminate individuals having excessive 5-HT biosynthesis, inhibited thrombocyte uptake or enhanced release of 5-HT, enhanced receptor-mediated vasoconstrictive responsiveness to 5-HT, or altered internalization of 5-HT by a specific transporter. Accordingly, it currently is our hypothesis that susceptibility to PHS is a consequence of an anatomically inadequate pulmonary vascular capacity combined with the functional predominance of vasoconstriction attributable to 5-HT over vasodilation and immune modulation attributable to NO. In

deed, studies focused on the fundamental basis for pulmonary hypertension in human patients repeatedly implicate 5-HT in the pathogenesis of idiopathic PAH (MacLean et al., 2000; Eddahibi et al., 2001; Marcos et al., 2003, 2004; Eddahibi and Adnot, 2005; Lawrie et al., 2005), PAH triggered by serotonergic appetite suppressant drugs (Seiler et al., 1974; Abenhaim et al., 1996; Eddahibi and Adnot, 2002; Naeije and Eddahibi, 2004), PAH initiated by hypoxia and monocrotaline toxin (Eddahibi et al., 1997, 1999, 2000; Marcos et al., 2003; Guignabert et al., 2005), and PAH associated with gram-negative sepsis, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and other inducers of secondary PAH in humans (Sibbald et al., 1980; Heffner and Repine, 1997; Egermayer et al., 1999; MacLean et al., 2000; Eddahibi et al., 2003).

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